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CYCLOSKRJABINIA TABORENSIS (LOEWEN, 1934), A CESTODE FROM THE RED BAT, *LASIURUS BOREALIS* (MÜLLER, 1776), AND A REVIEW OF THE FAMILY ANOPLOCEPHALIDAE

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Loewen (1934) described two strobilae from the intestine of the red bat, *Lasiurus borealis* (Müller, 1776), taken June 21, 1932, at Sterling, Kansas. The specimens were assigned to the anoplocephaline subfamily Linstowiinae and to the genus *Oochoristica* as *O. taborensis* n. sp. Spassky (1951) reproduced the figures of Loewen and designated *O. taborensis* as type of a new genus, *Cycloskrjabinia,* which he included in the subfamily Linstowiinae and family Linstowiidae. He (1949) had included the subfamilies Linstowiinae Fuhrmann, 1907, and Inermicapsiferinae López-Neyra, 1943, in the family Linstowiidae.

During the summer of 1958, Dr. Ralph E. Wheeler conducted a study of bats in New England to determine the presence and incidence of infection by the virus of encephalitis. The intestine of a silver-haired bat, *Lasionycteris noctivagens,* taken August 12, 1958, at Woods Hole, Massachusetts, contained the scolex of a recently excysted cestode, and a specimen of *Lasiurus borealis,* taken September 20, 1958, also at Woods Hole, contained two juvenile cestodes, mere cysticercoids just liberated from their cysts, and one complete strobila. These specimens were turned over to the writer for study and identification and grateful acknowledgment is made here to Dr. Wheeler. The juveniles were killed under coverglass pressure and all were fixed in Duboscq-Brasil fluid, stained, and mounted in damar.

External Morphology

The scolex from *L. noctivagans* measures 0.60 mm long and 0.43 wide; suckers oval, 0.11 by 0.13 mm in diameter. One of the excysted cysticercoids from *L. borealis* (fig. 6) is 0.63 mm long, 0.38 mm in greatest width, and the suckers are circular, 0.13 mm in diameter. The other scolex is much flattened; it is ovate, 0.73 mm long, 0.45 mm wide, and the sucker are oval, 0.15 mm long and 0.11 mm wide. Presumably these juveniles are conspecific with each other and with the strobilate specimen.

The complete worm (figs. 1, 2, 3) is mature; it consists of the scolex and 26 acraspedote proglottids and, as mounted, measures 45 mm in length. The maximum width is 2.00 mm. The scolex (fig. 4) is unarmed, without rostellum, 0.41 mm long, 0.35 mm wide, somewhat pointed anteriorly and somewhat concave posteriorly, with the anterior face of the first proglottid filling the concavity. The suckers are oval, 0.156 mm long and 0.11 mm wide. There is no neck; the first proglottid (fig. 4) is 0.12 mm long and 0.28 mm wide. The succeeding proglottids become longer and proglottid 8 is almost square. The following proglottids become increasingly elongate to number 15, which is 1.8 mm long and 0.76 mm wide. In these extended proglottids, at the level of the genital pore or immediately anterior to it, the proglottid is constricted, producing a vase-shaped outline. In more posterior and older, proglottids, contraction of longitudinal muscles caused the proglottids to become wider and thicker, with crenated and deeply indented sides. Proglottid 18 (fig. 5) is 1.38 mm long and 1.00 mm wide. Regression of the reproductive organs in proglottids 20 and 21 is followed by regression of the longitudinal and transverse muscles and the proglottids become merely thin-walled sacs filled with enormous numbers of eggs. The two terminal proglottids are much elongated; number 25 (fig. 3) is 8.2 mm long and 2.00 mm wide, while the last

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proglottid is 11.8 mm long and 1.74 mm in greatest width.

Internal Morphology

Since the worm was mounted entire, the study of internal anatomy was made by careful focussing and lacks the completeness and detail that could have been obtained by examination of serial sections. In proglottid 6, condensation of heavily staining cells denotes the position of the genital ducts and gives the first indication of the reproductive organs. The testes are recognizable in proglottid 10; they reach their greatest development in proglottids 15 to 18 and disappear in proglottids 20 or 21. Except for a central area that is occupied by the ovary, shell-gland, and the vitellaria, the testes extend from the level of the genital pore to a posterior limit about two-thirds of the distance from the vitellaria to the end of the proglottid. Anterior to the ovary there are about 50 follicles, spherical to oval to lobed, 0.036 to 0.062 mm in diameter, with more of them on the antiporal side of the proglottid. They are present as scattered strands at the sides of the ovary and the postovarian region contains 60 to 70 follicles. Ducts from the follicles unite to form a long vas deferens which extends in the midplane from the level of the ovary to that of the genital pore, where it is expanded, coiled loosely, and passes laterad to enter the cirrus-sac. The sac is oval, 0.13 to 0.16 mm long and 0.09 to 0.12 mm wide. The cirrus is 0.12 to 0.14 mm long and 0.05 to 0.064 mm wide, the shape is dependent on the extent of eversion. The genital pore is lateral, one-sixth to one-tenth of the length of the proglottid from the anterior end. The genital pores alternate irregularly and each opens from a small, shallow atrium. The cirrus-sac opens into the dorsal, anterior side of the atrium and the vagina opens into the posterior, ventral side. The vagina extends mediad along the posterior face of the cirrus-sac and the duct then curves posteriad, parallel to the vas deferens. The posterior portion is coiled as, filled with spermatozoa, it extends between the wings of the ovary to open into the oviduct near its origin from the ovary. Since only one worm was present in the bat, self-fertilization probably occurred. The ovary develops later than the testes and is first recognizable in proglottid 13. It is a two-winged structure, the wings diverging posteriorly, each composed of many slender lobes, situated at or near the middle of the proglottid. When fully developed, each wing is 0.18 to 0.25 mm wide and 0.24 to 0.38 mm long. The shell-gland, which measures 0.07 to 0.09 mm wide and 0.05 to 0.07 mm long, is situated in the interstice between and behind the posterior ends of the ovary. The vitellaria consist of two lobes, confluent in the midplane, and forming a gland which is spherical to oval, 0.30 to 0.35 mm in diameter, usually wider posteriorly and somewhat pointed anteriorly. The female reproductive organs regress and disappear in proglottids 20 or 21. There is no uterus; the eggs are dispersed throughout the parenchyma, with an estimated 6,000 to 9,000 eggs in each of the gravid proglottids. The eggs are spherical to oval, 0.036 to 0.040 mm in diameter and the onchospheres are 0.026 to 0.030 mm in diameter.

The excretory system comprises a pair of ducts on each side of the proglottid; the larger, ventral vessels are connected by a transverse bridge at the posterior end of each proglottid. The excretory ducts of the poral side pass dorsal to the genital ducts and the cirrus-sac.

This specimen shows substantial agreement with the account of Loewen and with the type specimen (now in poor condition) which was made available for study through the kindness of Dr. Allen McIntosh. The principal difference is in the presence of an unsegmented neck region in the type specimen and the complete absence of such an area in the worm from the bat taken in New England. Since the available material is so limited, the present worms are referred to *Cycloskrjabinia taborensis* and the strobilate specimen is deposited in the Helminthological Collection of the U. S. National Museum under the number 39,085.

DISCUSSION

The presence of the newly excysted juvenile worms is evidence of recently acquired infection and indicates that the intermediate host occurs in the Woods Hole area and is eaten in August and September. The finding of juvenile worms in late summer suggests that the infection of the intermediate host occurred either earlier in the same year or during the preceding autumn, and that the life-cycle is completed in New England. Whether or not the parasite will mature in *Lasionycteris noctivagans* is yet unknown. Discovery of mature cestodes in

Lasiurus borealis at Sterling, Kansas, and Woods Hole, Massachusetts, suggests a wide-ranging insect intermediate host. Although the bats are migrants, and the mature worms may have been acquired at some common wintering area, the finding of emerged cysticercoids in late summer would seem averse to such an hypothesis.

Stiles and Nolan (1931) compiled a key catalogue of parasites reported for Chiroptera. Among the cestodes, they listed a cysticercoid, *Taenia vespertilionis* Gmelin, 1790, and strobilate worms, *Taenia acuta* Rudolphi, 1819, and *Taenia obtusa* Rudolphi, 1819. This synopsis of literature is interesting and valuable but unfortunately the early descriptions are too incomplete to permit positive identification of species. Taxonomic clarification and validation of names can be accomplished only by recovery of new material from type hosts and type localities and redescription of the worms. Blanchard (1891a) transferred *T. acuta* to *Hymenolepis* and Macy (1931) devised a key to species of *Hymenolepis* found in bats.

The systematic position of *C. taborensis* is implicated in a taxonomic controversy. Loewen (1934) assigned the species to the genus *Oochoristica,* subfamily Linstowinae, family Anoplocephalidae. Historically, R. Blanchard (1891b) erected the subfamily Anoplocephalinae to contain the genera: *Anoplocephala* E. Blanchard, 1848; *Bertia* R. Blanchard, 1891; and *Moniezia* R. Blanchard, 1891. Cholodkowsky (1902) elevated the subfamily to family status. Fuhrmann (1907) expanded the family and arranged the genera in three subfamilies: Anoplocephalinae Blanchard, 1891, and two new subfamilies, viz, Linstowiinae and Thysanosominae. Among other features the subfamilies were distinguished by the form of the uterus: transverse tubular or reticular in the Anoplocephalinae; in dispersed parenchymatous capcules in the Linstowiinae; and by the formation of paruterine organs in the Thysanosominae. The Linstowiinae contained the genera *Linstowia* Zschokke, 1899, and *Zschokkea* Fuhrmann, 1902, whereas the Thysanosominae contained the genera *Thysanosoma* Diesing, 1835, and *Stilesia* Railliet, 1893. The name *Zschokkea* was preoccupied and Ransom (1909) renamed the genus *Zschokkeella,* but this name was also preoccupied and Fuhrmann (1921) named the genus *Multicapsiferina.* Meanwhile Gough (1911) erected a new genus, *Avitellina,* to contain *Taenia centripunctata* Rivolta, 1874, a species which Railliet had included in *Stilesia.* Gough removed *Avitellina* and *Stilesia* from the Thysanosominae and erected a new subfamily, Avitellinae, to receive them. Mola (1928) adopted the system of Fuhrmann (1907), but the next year he (1929) raised the subfamilies of Fuhrmann to family rank in a new tribe, Anoplocephalioidae. Fuhrmann (1930–1931), however, retained his earlier arrangement. Skrjabin (1933) proposed a suborder, Anoplocephalata, to contain two families: Anopolocephalidae, with the subfamilies Anoplocephalinae and Linstowiinae, and the family Stilesiidae, which he (1926) had erected to contain the subfamilies Stilesiinae and Thysanosominae. In Stilesiinae he included the genera *Stilesia* Railliet, 1893; *Avitellina* Gough, 1911; and *Thysaniezia* Skrjabin, 1926; while in Thysanosominae he included two genera, *Thysanosoma* Diesing, 1835, and *Ascotaenia* Baer, 1927. Since Spassky (1951) suppressed *Ascotaenia* as a synonym of *Avitellina,* there is no fundamental difference between the two subfamilies and the family Stilesiidae is virtually identical with the Thysanosominae.

Joyeux and Baer (1945) worked out the life-cycle of *Catenotaenia pusilla* (Goeze, 1782) Janicki, 1904, a parasite of *Mus musculus* that previously had been included in the families Taeniidae and Dilepididae, and showed that the intermediate host is a tyroglyphid mite, *Glyciphagus domesticus.* They listed developmental and morphological features which, they predicated, justified inclusion of *Catenotaenia* in the subfamily Anoplocephalinae. Spassky (1949) adopted the idea of Joyeux and Baer and proposed a new subfamily, Catenotaeniinae, in the family Anoplocephalidae. Spassky (1950a, 1950b) made a revision of the anoplocephaline cestodes to express what he conceived to be phylogenetic sequence and the system which was presented in detail in his (1951) monograph was repeated in his (1959) systematic outline. In his arrangement, Spassky recognized the suborder Anoplocephalata Skrjabin, 1933, superfamily Anoplocephaloidea Spassky, 1949 (= Anoplocephalioidae Mola, 1929), with four famlies: Anoplocephalidae Cholodkowsky, 1902; Avitellinidae Spassky, 1950; Linstowiidae (Mola, 1929) Spassky, 1949; and Catenotaeniidae Spassky, 1950. The family Catenotae-

niidae contained two genera: *Catenotaenia* Janicki, 1904 and *Skrjabinotaenia* Akhumian, 1946. The latter genus had been erected to contain *Catenotaenia lobata* Baer, 1925, and *Catenotaenia oranensis* Joyeux and Foley, 1930, but according to Wolfgang (1956) *Skrjabinotaenia* is identical with *Catenotaenia*.

The family name Avitellinidae was admittedly a deliberate renaming of the Family Thysanosomidae Mola, 1929, which had been recognized by Skrjabin and Schulz (1937). Indeed, except for the inclusion of a new family, Catenotaeniidae, the system of Spassky is virtually identical with that of Mola (1929). But the erection of the family Avitellinidae Spassky, 1950, was quite illegal, since it contained the genus *Thysanosoma* and the subfamily Thysanosomatinae. *Thysanosoma* was the previously validly named type of the subfamily and family to which it was assigned. In his subsequent publications (1951) and (1959), Spassky maintained the family Avitellinidae with the subfamilies: Avitellininae Gough, 1911; Thysanosomatinae Fuhrmann, 1907; and Thysanieziinae Skrjabin and Schulz, 1937. The rules of zoological nomenclature provide: "The name of the family is formed by adding the ending *idae*, the name of the subfamily by adding *inae*, to the root of the name of the type genus." What Spassky has done is to select a genus other than *Thysanosoma* as type of the family and change the family name from Thysanosomatidae to Avitellinidae. This procedure was analyzed and proscribed in a statement by C. W. Stiles, Secretary of the International Commission on Zoological Nomenclature, published in Stunkard and Nigrelli (1930).

A further unwarranted and illegal action was taken by Spassky (1954) in the erection of a new genus, *Vampirella*, to contain *Hymenolepis skrjabinariana* Skarbilovitsch, 1946, from the bat, *Eptesicus turcomanus*, and other species from bats and rodents. In the new genus, Spassky included *Milina grisea* van Beneden, 1873. The genus *Hymenolepis* is a large and cumbersome group and the removal of certain species is undoubtedly justified, but if the species *skrjabinariana* and *grisea* are congeneric as predicated by Spassky, the name of the genus is *Milina* van Beneden 1873, and *Vampirella* is a synonym. Yamaguti (1959) included several other species in *Vampirella;* these species were removed from other genera, chiefly *Hymenolepis* and *Dicranotaenia*. The distinguishing features of these genera are not clearly manifest but if *Vampirella* is a synonym of *Milina*, certain of the species must be transferred to *Milina*.

Wardle and McLeod (1952) independently erected the family Catenotaeniidae for the single genus *Catenotaenia*, but otherwise they followed the system of Fuhrmann (1931) and included three subfamilies, viz, Anoplocephalinae, Linstowiinae, and Thysanosominae, in the family Anoplocephalidae.

In the family Linstowiidae, Spassky (1951) included two subfamilies: Linstowiinae Fuhrmann, 1907, and Inermicapsiferinae López-Neyra, 1943. Dollfus (1954) made no comment on the inclusion of the Inermicapsiferinae in the Linstowiidae but questioned the integrity of the Linstowiinae as set up, consisting of ten genera: *Linstowia* Zschokke, 1899; *Atriotaenia* Sandground, 1926; *Cycloskrjabinia* Spassky, 1951; *Mathevotaenia* Akhumian, 1946; *Multicapsiferina* Fuhrmann 1921; *Oochoristica* Lühe, 1898; *Oschmarenia* Spassky, 1951; *Pancerina* Fuhrmann, 1899; *Semenoviella* Spassky, 1951; and *Sobolevina* Spassky, 1951. Dollfus observed that the genus *Oochoristica* was restricted to species from amphibians and reptiles and that the species from birds and mammals that formerly were included in *Oochoristica* were transferred to other genera. He noted that the allocation of species to genera was made largely on their distribution among vertebrate hosts, e.g., the parasites of birds were assigned to *Multicapsiferina* and *Sobolevina;* the single species from a bat was made type of a new genus, *Cycloskrjabinia;** but *Mathevotaenia* contained species from marsupials, edentates, insectivores, rodents, carnivores and primates.

* A second species of *Oochoristica* from North American bats was described by Marietta Voge (1954, Tr. Am. Micr. Soc. **73**: 404–407) as *O. antrozoi*, but she stated that it bears no resemblance to *O. taborensis* Loewen, 1934. Yamaguti (1959, Systema Helminthum) transferred the species *antrozoi* to the genus *Mathevotaenia* Akhumian, 1946. Della Santa (1956, Rev. Suisse Zool. **63**: 1–113) noted that species of *Oochoristica* infect reptiles and mammals in the warm regions of the earth. He suppressed both *Cycloskrjabinia* and *Mathevotaenia* as synonyms of Oochoristica.

The type species of *Mathevotaenia* is *Catenotaenia symmetrica* Baylis, 1927, a species which had been transferred to *Oochoristica* by Meggitt and Subramanian (1927) and redescribed as *O. ratti* n. sp., by Yamaguti and Miyata (1937). Lynsdale (1953) proposed a new genus, *Meggittina,* to contain a new species, *M. baeri,* from house and granary rats in Rhodesia.

Baer and Fain (1955a) adopted the system of Fuhrmann (1907) but these authors (1955b) made significant changes in taxonomy. The genus *Inermicapsifer* Janicki, 1910, erected to contain *Taenia hyracis* Rudolphi, 1808, as type, was transferred from the Linstowiinae to the family Davaineidae and subfamily Davaineinae. According to Stiles and Hassall (1912), the type species is identical with *Taenia caviae-capensis* Pallas, 1767, and other names are listed as synonyms by Yamaguti (1959). Another genus, *Thysanotaenia* Beddard, 1911, erected to contain *Thysanotaenia lemuris* Beddard, 1911, a parasite of *Lemur varius* and *L. macao,* and included in the Linstowiidae by Spassky (1951), was also transferred by Baer and Fain (1955b) to the family Davaineidae. The genus *Multicapsiferina* Fuhrmann, 1921, erected to contain *Taenia linstowi* Parona, 1855, after *Zschokkea* and *Zschokkeella* were found to be preoccupied, was suppressed as a synonym of *Inermicapsifer* when the specimens of Parona were found to be identical with *T. hyracis* Rudolphi, 1808. The genus *Sobolevina,* erected by Spassky (1951) to receive *Inermicapsifer otidis* Meggitt, 1927, was suppressed when the type species, *S. otidis,* was transferred to the genus *Ascometra* Cholodkowsky, 1912, a member of the family Dilepididae.

After study of material of several species of *Catenotaenia* and the type material of *Meggittina baeri* Lynsdale, 1953, Wolfgang (1956) declared that *Meggittina* is a synonym of *Catenotaenia* and the species *M. baeri* was transferred to that genus. Wolfgang also suppressed *Skrjabinotaenia* as a synonym of *Catenotaenia* since, in his opinion, the characters proposed to separate them proved to be unreliable. He noted that Spassky (1951) and Wardle and McLeod (1952) independently proposed the family Catenotaeniidae but agreed with Lynsdale (1953) that the reasons given by Wardle and McLeod for erecting the family Catenotaeniidae parallel those given by Joyeux and Baer (1945) for including the genus in the family Anoplocephalidae. He further agreed with Lynsdale that separation of *Catenotaenia* from the Anoplocephalidae serves no useful purpose, but admitted some merit in retaining the subfamily Catenotaeniinae Spassky, 1949.

The morphological basis for classification of the anoplocephaline cestodes is supplemented by information derived from studies on life cycles. Stunkard (1934) analyzed the problem of the life history; from bionomic and experimental data he predicated the necessity for an intermediate host and delineated the characteristics of that host with clarity and accuracy. He (1937) announced that *Moniezia expansa* of cattle, sheep, and other ruminants is transmitted by oribatid mites and (1939a) described and figured the developmental stages in the intermediate host. The transmission of *M. expansa* by oribatids was followed by the demonstrations that (1939b) *Cittotaenia ctenoides* of rabbits and (1940) *Bertiella studeri* of primates are also transmitted by oribatid mites. Further information on the life cycles of cestodes from hares and rabbits was presented (Stunkard, 1941). It was affirmed that many different species of orbatid mites could serve as intermediate hosts of each of the cestode species. The discovery of the life cycle of *M. expansa* was quickly confirmed in the United States and in foreign countries. Stoll (1938) in America, Pullar (1939) in Australia, and Potemkina (1941) in Russia repeated the experiments with positive results. Baskirova (1941a, 1941b) reported that oribatid mites serve as intermediate hosts of the equine cestodes, *Anoplocephala perfoliata, Anoplocephala magna,* and *Paranoplocephala mamillana.* Potemkina (1944a) reported that *Moniezia benedeni* and (1944b) that *Thysaniezia ovilla* are transmitted by various species of oribatid mites. Kates and Runkel (1948) compiled a summary of published information on sixteen genera and eight families of oribatid mites then known as intermediate hosts of anoplocephaline cestodes. Freeman (1952) showed that *Monoecocestus variabilis* and *Monoecocestus americanus,* parasites of the Canadian porcupine, *Erethizon dorsatum* (Linnaeus, 1758), are transmitted by 14 species representing 12 genera of oribatid mites and Dorothy Melvin (1952) reported the life cycle of a twelfth species of anoplocephaline cestodes transmitted by oribatids; she found that *Monoecocestus sigmodontis* (Chandler and Suttles,

1922), a common and widely distributed parasite of cotton rats, developed in *Protoschelobates seghetti* and in four other species, all in different genera.

As noted, Joyeux and Baer (1945) showed that *Catenotaenia pusilla* is transmitted by tyroglyphid mites and Spassky (1949) named *Catenotaenia* as type of a new subfamily in the family Anoplocephalidae. The following year he (1950a) elevated the subfamily to family status and independently Wardle and McLeod (1952) proposed a family Catenotaeniidae. Yamaguti (1959) accepted the family Catenotaeniidae with two genera, *Catenotaenia* and *Skrjabinotaenia,* the latter of which is very similar to *Catenotaenia* and according to Wolfgang (1956) a subjective synonym. As noted by Joyeux and Baer, *Catenotaenia* agrees essentially with the diagnosis of the family Anoplocephalidae, and while it can not be included in the subfamily Anoplocephalinae as predicated by them, it should be included in the subfamily Catenotaeniinae Spassky, 1949.

Rentdorff (1948) reported that *Oochoristica ratti* Yamaguti and Miyata, 1937 [=*Mathevotaenia symmetrica* (Baylis, 1927) Akhumian, 1946], developed in six different tenebrionid and dermestid beetles and in the Indian meal moth, *Plodia interpunctella.* Milleman and Read (1953) found that an undescribed species of *Oochoristica* from kangaroo rats and *Oochoristica scleroporus* Voge and Fox, 1950 from the Pacific fence-lizard, *Scleroporus occidentalis,* developed in larvae and adults of *Tribolium confusum* and the worms from the kangaroo rats developed also in the tenebrionid beetle, *Gnathocerus cornutum.* They stated (p. 29): "It has been amply demonstrated that oribatid mites are intermediate hosts of anoplocephaline cestodes. The present findings strongly indicate that the linstowiine cestodes develop in coleopterus insects. These gross differences in biology, along with the recognized anatomical differences between the two groups, justify the recognition of the family Linstowiidae." Milleman (1955) identified the kangaroo rats as *Dipodomys merriami* and *D. panamintinus,* and described the cestode as *Oochristica deserti.* Cysticercoids developed in the adult and larval stages of various coleopteran and lepidopteran insects and infected *D. merriami, D. panamintinus, Citellus leucurus, Perognathus longimembris,* and *Mesocricetus auratus.* Presumably *Oochoristica deserti* is identical with *Catenotaenia californica,* which had been described by Dowell (1953) from the same host and locality.

Gallati (1959) described the successive stages in the life-history of *Atriotaenia* (*Ershovia*) *procyonis* (Chandler, 1942) Spassky, 1951, a species originally described as *Oochoristica procyonis* from the raccoon, *Procyonis lotor.* The larval stages developed in the flour beetle, *Tribolium castaneum,* and were almost identical with those of *Oochoristica ratti* and *Oochoristica deserti* as described by Rendtorff (1948) and Milleman (1955).

The genus *Atriotaenia* was erected by Sandground (1926) to contain a new species, *A. parva,* from *Nasua nasua* in Brazil. Meggitt (1934) suppressed *Atriotaenia* as a synonym of *Oochoristica* and since *Oochoristica parva* (Sandground, 1926) Meggitt, 1934, antedates *O. parva* Baylis, 1929, from the Javanese lizard, *Lygosoma chalcides,* he declared that the latter species should be renamed. Actually the species was named, *Oochoristica lygosomatis* by Skinker (1935). Baer (1935) removed *Raillietina* (*Skrjabinia*) *parva* (Janicki, 1904) (syn. *Davainea parva* Janicki, 1904), to the genus *Oochoristica* and noted that three different species had been designated *O. parva.* He maintained the name for the species described by Janicki (1904) which had priority and proposed the name *O. sandgroundi* for the species described by Sandground, and *O. baylisi* for the species described by Baylis (1929). Spassky (1951) named *O. sandgroundi* Baer, 1935, as type of the restored genus *Atriotaenia* which he divided into two subgenera, *Atriotaenia* and *Ershovia,* distinguished by the larger and more muscular genital atrium in the former. The restoration of *Atriotaenia* as a valid genus automatically restores validity to the type species, *A. parva,* and *A. sandgroundi* disappears in synonymy. Spassky (1951) also recognized *Mathevotaenia* Akhumian, 1946, with *M. symmetrica* (Baylis, 1927) Akhumian, 1946 (syn. *Oochoristica ratti* Yamaguti and Miyata, 1937) as type species. However, the characters named to distinguish between *Mathevotaenia* and *Atriotaenia* are so tenuous and variable that the two generic concepts merge and *Mathevotaenia* should be suppressed as a synonym of *Atriotaenia.*

In a research note, Allen (1959) reported that the fringed tapeworm, *Thysanosoma acti-*

nioides Diesing, 1834, is transmitted by psocid barklice or booklice, *Liposcelis bostrychophilus*, and an undescribed species of *Rhyopsocus* (Psocoptera: Corrodentia). Larval stages, including a fully formed cysticercoid with motile invaginated scolex, were recovered from these insects after feeding them eggs of the cestode.

Yamaguti (1959) recognized the family Catenotaeniidae and the family Anoplocephalidae with five subfamilies: Anoplocephalinae, Inermicapsiferinae, Linstowiinae, Rajotaeniinae, and Thysanosomatinae. As noted earlier, Baer and Fain (1955b) removed *Inermicapsifer* from the Anoplocephalidae and included it in the Davaineidae. The subfamily Rajotaeniinae was proposed by Yamaguti (1959) to contain a single species, *Rajotaenia gerbilli* Wertheim, 1954. Spasski (1955) had suppressed *Rajotaenia* as a synonym of *Skrjabinotaenia* and Wolfgang (1956) suppressed *Skrjabinotaenia* as a synonym of *Catenotaenia*.

Gibbs (1960) reported *Avitellina arctica* Kolmakov, 1938, from the caribou, *Rangifer arcticus*, in northern Canada, the first record of the genus in the western hemisphere. The redescription of the species shows its close resemblance to *A. centripunctata* and Gibbs included *Avitellina* in the subfamily Thysanosomatinae, family Anoplocephalidae.

It is clearly evident that the family Anoplocephalidae constitutes a coherent entity and that it contains four distinct morphological and bionomic groups which can be recognized as subfamilies: (1) Anoplocephalinae Blanchard, 1891, whose members are transmitted by oribatid mites; (2) Catenotaeniinae Spassky, 1949, transmitted by tyroglyphid mites; (3) Linstowiinae Fuhrmann, 1907, transmitted by coleopterous and lepidopterous insects; and (4) Thysanosomatinae Fuhrmann, 1907, transmitted by psocopterous insects. A number of the genera proposed in recent years are of doubtful validity, but the genus *Cycloskrjabinia* Spassky, 1951, appears to be a distinct member of the Linstowiinae since the testes are distributed both anterior and posterior to the ovary. Since bats are insectivorous, it is also likely that some flying insect serves as the intermediate host.

Summary

A second infection of the bat, *Lasiurus borealis*, by the cestode, *Cycloskrjabinia taborensis* (Loewen, 1934), is reported. The taxonomy of the family Anoplocephalidae is reviewed. The erection of the family Avitellinidae by Spassky (1950a) and of the genus *Vampirella* by Spassky (1954) violate the International Rules of Zoological Nomenclature and both are invalid. *Mathevotaenia* Akhumian, 1946, is suppressed as a synonym of *Atriotaenia* Sandground, 1926. With the suppression of *Sobolevina* Spassky, 1951, as a synonym of *Ascometra* Cholodkowsky, 1912, and the removal of *Thysanotaenia* Beddard, 1911, and *Inermicapsifer* Janicki, 1910, from the Linstowiinae to the family Davaineidae by Baer and Fain (1955b) the family Linstowiidae of Spassky is reduced to the subfamily Linstowiinae. The suppression of *Skrjabinotaenia* Akhumian, 1946, as a synonym of *Catenotaenia* Janicki, 1904, by Wolfgang (1956) leaves the family Catenotaeniidae Spassky, 1950a, with a single genus and since the worms are clearly anoplocephaline cestodes, the group merits no more than subfamily status. On both morphological and bionomic grounds, the family Anoplocephalidae is maintained with four subfamilies: Anoplocephalinae Blanchard, 1891; Catenotaeninae Spassky, 1949; Linstowiinae Fuhrmann, 1907; and Thysanosomatinae Fuhrmann, 1907. There is at present no virtue in attempts to elevate these subfamilies to family status in a superfamily or suborder. When more information is available, it may be feasible to recognize suprafamilial relations in the Cyclophyllidea.

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EXPLANATION OF PLATE I

Camera lucida drawings of *Cycloskrjabinia taborensis;* figures 1, 2, and 3 are drawn at the same magnification.

FIGURE 1. The fist 21 proglottids. (Proglottid 18 is left blank but is shown in figure 5. In proglottid 20, the gonads are indistinct and the parenchyma contains many eggs.)

FIGURE 2. Proglottids 22, 23, and 24. (Contain developing eggs; muscles contracted to form crenated walls of proglottids.)

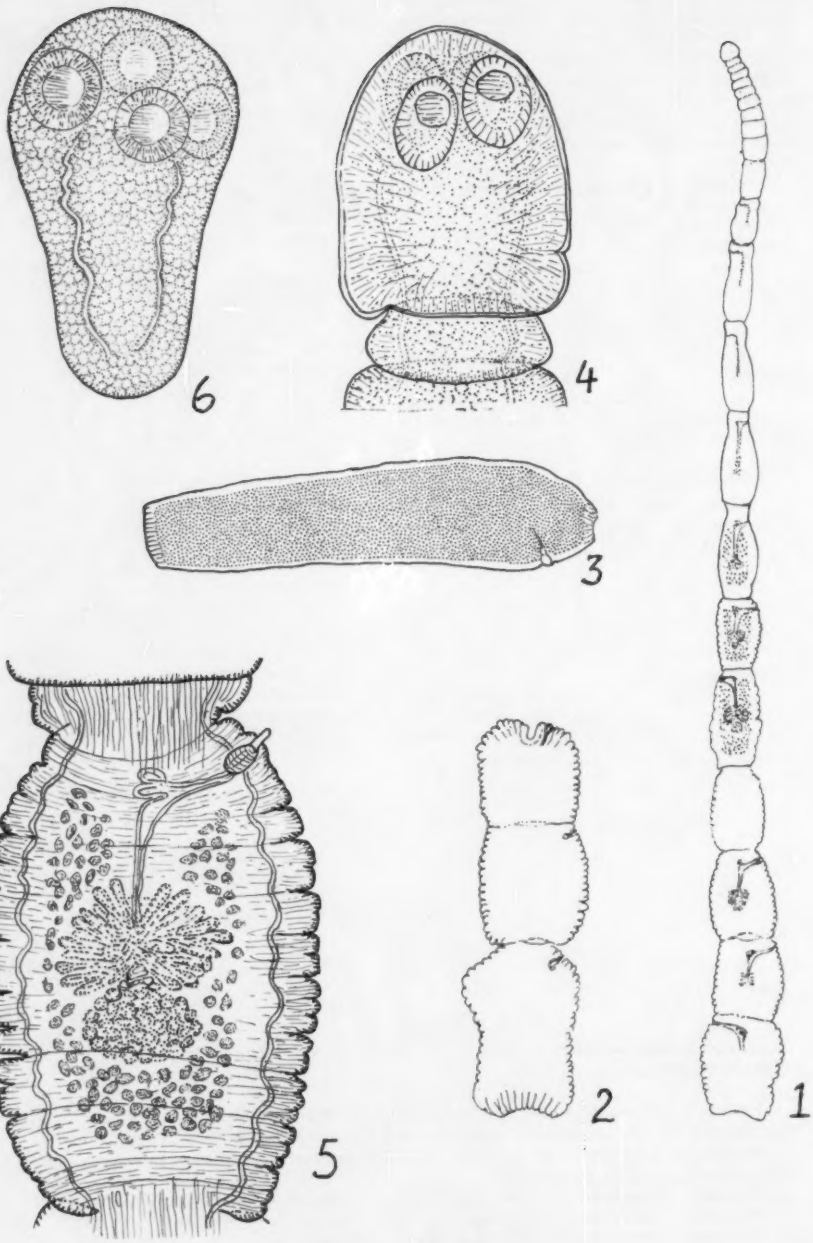
FIGURE 3. Proglottid 25. (Muscles relaxed; proglottid saccate, 8.2 mm long.)

FIGURE 4. Scolex and first proglottid. (No neck region; scolex 0.41 mm long.)

FIGURE 5. Proglottid 18. (Fully mature; 1.38 mm long.)

FIGURE 6. Recently excysted cysticercoid. (0.63 mm long.)

PLATE I



EPIZOOTIOLOGICAL AND EXPERIMENTAL STUDIES ON BOVINE CYSTICERCOSIS IN EAST AFRICA

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The control of most of the major diseases of cattle on the African continent, together with increased interest in medical and veterinary public health, has focused attention on diseases which although less spectacular in their manifestations are nevertheless of considerable importance to the health and economy of the community. Bovine cysticercosis is one such disease and, although inadequate meat inspection facilities make it difficult to ascertain the exact incidence in many areas, the condition is widespread and extremely common. Some typical figures are: Bechuanaland 0.6 to 86 percent (Rep. Vet. Serv. 1956); Belgian Congo 22 to 60 percent (Rep. Vet. Serv. 1957); Cameroons 16 percent (Merle, 1958); Ethiopia 80 percent (Graber, 1959); French Guinea 20 percent (Graber, 1959); Kenya 30 percent (Ginsberg et al, 1956); Madagascar 2 to 17 percent (Rapp. Ann., 1956); Nigeria 10 percent (Graber, 1959); Oubangui 30 to 69 percent (Graber, 1959); Sierra Leone 23 to 55 percent (Peel, 1953); Tanganyika 50 percent (Rep. Vet. Serv. 1958); Tchad Republic 0.4 to 15 percent (Graber, 1959); Uganda 5 to 75 percent (Coyle, personal communication 1960); Union of South Africa 2.8 percent (Merle, 1958). The status of other African countries was reviewed by Graber (1959), Merle (1958), and Viljoen (1937).

Adequate meat inspection facilities, thorough cooking of meat and the desire of infected persons to seek treatment have resulted in the virtual eradication of bovine cysticercosis in civilized communities. In contrast, meat inspection facilities are lacking over large areas of Africa, meat is often consumed raw or partially cooked and infected persons frequently regard their tapeworms with equanimity. While the situation will be resolved eventually by the expanding medical, veterinary, and educational services, the present high incidence of cysticercosis is a considerable source of economic loss in areas such as East Africa in which beef is commercially produced on a large scale. This is illustrated by a survey conducted from 1953–56 at the largest abattoir in East Africa by *Ginsberg, Cameron, Goddard and Grieve* (1956). They examined 42,500 cattle reared on European-owned farms and 12,850 African-bred cattle and, on a basis of routine meat inspection incisions, detected cysts of *C. bovis* in 30.6 percent of European-owned and 29.4 percent of African-owned stock. More than 20 cysts were found in 8.3 percent of the European stock and in 19.9 percent of the African stock. The economic implications included the complete condemnation of all carcasses with over 20 cysts and the cost of deep-freezing carcasses with up to 20 cysts for several weeks, these being subsequently retailed at reduced prices.

This paper describes an attempt to elucidate the epizootiology of bovine cysticercosis in East Africa. Information on this aspect of bovine cysticercosis is non-existent or negligible in territories where the disease is enzootic, and in the past has been deduced entirely from observations on mature animals or in calves during meat inspection.

MATERIALS AND METHODS

Calves and Cattle

Kenya: Over a period of 18 months, 122 calves were obtained from farms within a radius of 60 miles of Nairobi. The majority were Friesian, Ayrshire, or Jersey; the remainder, Boran. The calves were isolated in the laboratory pens within 24 hours of birth, 22 having received no colostrum. The calves were reared entirely indoors, fed on milk and calf meal, and bedded on sawdust. Calves older than 3 weeks received hay sterilized by steam. Monthly fecal samples from the two stockmen attending the calves and the two men responsible for the cows supplying the milk were consistently negative for *T. saginata eggs*. After laboratory hours and during lunch the calf pens were locked to preclude the risk of infection from other employees.

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A further eight calves reared on three of the farms were brought to the laboratory when 24 to 42 days old. They were killed and autopsied immediately on arrival. In addition 31 calves born out-of-doors and suckled by their dams on the laboratory farm were autopsied. Ten of them were unhandled from birth until death apart from ear-tagging.

Adult Cattle: Seventy cattle of Boran or European breeds aged 18 months to aged were used. Almost all were males. These had been raised commercially and brought to the laboratory grazing area several months previously. *T. saginata* eggs were administered to 48 of the cattle, the remaining 22 serving as controls.

Pregnant Cows: Ten pregnant cows obtained from the laboratory herd were maintained on grass throughout the whole experiment. The dams and calves were unhandled apart from ear-tagging the calves.

Scotland: In Glasgow, seven Ayrshire calves were reared at the University Veterinary School until 14 weeks of age when each was infected with *T. saginata* eggs. On another occasion, five bovines aged 3 months to adult were infected. These eggs were obtained from the same sources, although not from the same samples, as those used for the East African calves and sent air-post to Scotland. The calves and cattle were killed and autopsied 20 to 32 days after infection. Because of the relative rarity of taeniasis in humans in Scotland, no special precautions were taken in rearing the calves.

Experimental Infection

Calves: A number of the calves at various ages after birth were dosed by bottling with a suspension of *T. saginata* eggs in normal saline. Each calf received a single dose of 10,000 to 20,000 eggs unless otherwise stated. The eggs were obtained from naturally expelled segments collected from the local dispensary and were used 3 days to 3 weeks after collection. Eggs from several "donors" were always present in any given dose and the calves were infected in groups of 4 to 10; in groups of this size at least one heavily infected calf was always found at autopsy, thus eliminating criticism of egg infectivity.

Adults: These were infected in groups of 6 to 12 each animal receiving a single dose of eggs orally, by stomach tube, or by intra-ruminal injection. The doses ranged from 500 to 150,000 eggs. At the same time as each group of animals was infected a number of 1-day-old calves was also infected with eggs from the same batch. When these calves were killed and autopsied 14 days later, heavy infestations were always found in one or more of the number, thus providing proof of egg infectivity.

Autopsy

The calves and adults were autopsied at intervals ranging from 12 to 55 days after infection and most frequently at 14 days. The dams and their calves were autopsied 10 to 29 days after calving. All carcass muscles were cut into small pieces and examined for cysts. The thoracic and abdominal viscera, particularly the heart, lungs, liver and kidneys, were also checked.

Examination of selected cysts was made by gross dissection of the host-capsule and enucleation of the developing cysticercus; this was measured intact on a slide and was then flattened by a coverslip and studied morphologically. Other cysts were fixed in formal saline, dehydrated, cleared, and embedded in paraffin wax; the sections made from them were stained with haematoxylin and eosin. The number of *T. saginata* eggs per gram of feces in a series of human patients were estimated by the McMaster slide technique (Gordon and Whitlock, 1939) or by the Stoll method (Stoll, 1930).

Criteria of Experimental Infection

The number of cysts in each animal was counted; or, if numerous, estimated according to the following scheme:

(1) ++++ Many hundreds to thousands of cysts.
(2) +++ Several hundred cysts.
(3) ++ 41 to 100 cysts.
(4) 0, + 0 to 40 systs.

Field Experiment

Eighty Boran and cross-bred calves ranging from 2 weeks to 9 months of age were autopsied. The calves were drawn from 12 farms in representative beef-raising areas in Kenya and Uganda and included 7 European-owned farms using African labor, 3 African farms, 1 African cooperative ranching project, 1 government stock farm and one laboratory ranch in Uganda. Great difficulty was experienced in persuading African farmers to sell suckling calves.

Forty of these calves were experimentally infected. Each received 10,000 *T. saginata* eggs and was killed about 14 days after infection. Almost always calves were infected and autopsied on their farms of origin. The infectivity of the eggs was checked by the production of successful experimental infections in calves reared at the laboratory.

RESULTS

1. PRENATAL AND EARLY POST-NATAL INFECTION IN CALVES

The experiments reported in this section are concerned with the occurrence of naturally acquired cysticercosis in very young calves and were stimulated by the frequency with which light infections of cysticercosis were observed at autopsy in young experimental calves reared at the laboratory for other purposes. The study also includes the results of experiments in which pregnant cows were given *T. saginata* eggs in an attempt to infect their calves prenatally.

Cysticercosis in Calves Brought to the Laboratory

Thirty-three calves were brought to the laboratory and reared and autopsied as de-

scribed in the section on materials and methods. The number of cysts found and the age of the calves at autopsy are shown in table I.

TABLE I. *Numbers of calves infected with cysticercosis when reared from shortly after birth in a helminth-free environment.*

Age of calf in days when killed	Farm of origin	Number of cysts at autopsy
2	G	Negative
3	D	"
3	A	"
5	E	"
5	G	"
7	D	"
10	C	"
10	C	"
10	C	"
12	B	"
15	A	"
16	A	"
16	D	3
17	D	7
18	D	Negative
25	B	1
25	B	20
25	G	17
25	A	39
25	D	2
26	E	18
27	D	7
28	G	19
28	B	1
32	D	17
32	G	7
52	D	12
33	D	4
36	G	Negative
38	C	6
48	G	Negative
52	A	6
55	C	2

Size and appearance of the cysts in the 18 infected calves were almost always related to the age in days of the calf when killed. The 16-day-old calf, the youngest in which cysticercosis was found, had three cysts whose host-capsules measured 2 to 4 mm in diameter; it was not possible to dissect out the cysticerci because of their minute size. The host-capsule of the 25 to 35-day-old group ranged from 5 to 10 mm in diameter. On gross dissection some were composed of firm white connective tissue with a central cavity containing the cysticercus, while other consisted of a fibrous sac enclosing necrotic or purulent material from which the cysticercus could usually be expressed. The cysticerci from these measured 0.5 to 2.5 mm in diameter. Invagination of the developing scolex was commonly noted although suckers were not present. Cysticerci were not found in some cysts despite careful search.

The two oldest infected calves were killed at 52 and 55 days of age respectively. In the heart of the former were 6 hard fibrous lesions 2 to 3 mm in diameter in which cysticerci were not found; the latter had two typical mature cysticerci about 8 mm in diameter whose scoleces possessed suckers. The one exception to the relationship between age of calf and size of cysts was a calf killed at 17 days of age in which 7 cysts were found; six of these were within the usual limits while the seventh was 9 mm in diameter and contained a cysticercus 0.75 mm in diameter.

Cysticercosis in Calves Reared on the Supplying Farms

Eight calves reared on three of the supplying farms until 24 to 42 days of age were killed and autopsied immediately on arrival at the laboratory. Four of the calves were infected; two of these contained 20 to 30 cysts up to 12 mm diameter in each carcass; each of the other two calves had 1 cyst 5 mm diameter.

Attempts to Produce Cysticercosis Prenatally

T. saginata eggs were administered orally or intravenously to each of eight pregnant cows as shown in Table II. While the eggs given to seven of the cows were untreated in any way, some of the eggs injected intravenously into cow 8 were previously treated with hatching solutions described by Silverman (1954). Our experience with these solutions has been described previously (Urquhart, 1958) and the motility of the hatched oncospheres in this instance was about 2 percent. The cows and their calves were maintained as described in materials and methods. A further two cows and their calves were maintained similarly except that they were not experimentally infected.

The results (Table II) show that none of the calves was infected with cysticercosis at autopsy. Of the 10 cows, 2 were infected with mature cysticerci and one with a calcified cysticercus; none of these cysticerci had developed from our experimental infections. The cow infected intravenously with the hatched oncospheres possessed two developing cysticerci which could have originated from the experimental infection.

Cysticercosis in Unhandled Suckled Calves

Ten calves born on the laboratory farm and unhandled until death were autopsied at 13, 14, 14, 18, 18, 20, 21, 21, 22 and 24 days old, respectively. All were shown to be completely free from cysticerosis.

TABLE II. *The attempted production of prenatal infection in calves by the administration of* T. saginata *eggs to pregnant cows.*

No. of Cow	Size and route of infection	No. of days prior to calving	Age of calf in days when killed	No. of cysts found at autopsy	
				Calf	Cow
1	35,000 eggs orally	62	20	Negative	Negative
	50,000 eggs orally	7			
2	50,000 eggs orally	42	25	Negative	Negative
3	35,000 eggs orally	35	10	Negative	40 mature cysts
4	16,000 eggs orally	20	29	Negative	Negative
5	40,000 eggs orally	18		Negative	Negative
	70,000 eggs orally	15	14	Negative	Negative
6	50,000 eggs intravenously	101	28	Negative	Negative
7	50,000 eggs intravenously	24	25	Negative	Negative
	70,000 eggs intravenously				
8	120,000 eggs, treated with pepsin and trypsin, intravenously	14	23	Negative	One 8-mm lesion with developing cysticercus 1.5 mm dia. One 6-mm lesion, no cysticercus
9	Untreated	—			
10	Untreated	—			
			25	Negative	6 mature cysts
			21	Negative	1 calcified cyst

2. EXPERIMENTAL CYSTICERCOSIS IN CALVES AGED 1–40 DAYS

Young calves hand-reared in the laboratory farm within a day of birth in the environment described in materials and methods were used. No attempt was made to eliminate the postnatal infections described in the previous section since this appeared to be a characteristic feature of bovine cysticercosis in East Africa. Instead, the experiments were designed so that it was possible to compare the results obtained with uninfected calves with those of calves naturally infected during the first day of life. A preliminary report of part of this work appeared earlier (Urquhart, 1958).

The Proportion of Calves Resistant to Experimental Infection

A single dose of *T. saginata* eggs was administered to each of the 66 calves. The autopsy results showed that 27 calves were resistant to the experimental infection as judged by the criteria previously outlined. In 39 of the calves the sizes of the cysts conformed to a regular pattern. Irrespective of the numbers of cysts present their size and appearance was similar for any stated length of infection. Thus, in calves killed 14 days after infection, the most frequent time of autopsy, the host-capsules ranged from 1.5 mm to 4 mm in diameter and it was not possible to dissect out the cysticerci because of their minute size. These cysts were considered to have been derived from the experimental infection.

In 26 of the remaining 27 calves a small number of relatively large cysts was found in each of the carcasses in addition to the variable number derived from the experimental infection. These large cysts whose sizes ranged from 6 to 12 mm and which usually contained developing cysticerci of 0.5 mm to 3 mm diameter were similar to those found in calves naturally infected with cysticercosis in the first day or so of postnatal life except that in the few calves autopsied after 7 weeks of age the invaginated scoleces also possessed four developing sucklers. The number of these cysts in any one carcass ranged from 1 to 60. Figure 1 shows a piece of heart muscle removed from a 28-day-old calf which had been infected 14 days previously. A number of small cysts from the 14-day infection can be seen together with a single large cyst of 10 mm in diameter. In the 27th calf 2 cysts 6 mm diameter were found each containing a cysticercus of 0.75 mm in diameter. Since this calf was experimentally infected a few hours after birth and killed 16 days later, these cysticerci were larger and more developed than one would normally expect.

For convenience of description in the remainder of this paper an experimentally infected calf shown at autopsy to have been naturally infected previously will be termed a "preinfected" calf as opposed to a "normal" calf.

The role of Preinfection in the Production of Resistance to Experimental Infection

The susceptibilities of the preinfected and normal calves to experimental infection were compared. Ten of the 39 normal calves (26

percent) and 17 of the 27 preinfected calves (63 percent), were resistant.

Previous infection, therefore, conferred a significant resistance to a subsequent experimental infection (chi-square, 7.711, $P<0.01>0.001$). Of the 17 calves described as preinfected and resistant, 8 were highly resistant in that only 0, 0, 0, 2, 6, 6, 9 and 10 cysts, respectively, developed from the experimental infection; each of these carcasses possessed an average of 8 large cysts derived from a previous natural infection.

Of the 10 calves classified as normal and resistant, 4 were completely negative at autopsy; all 4 came from a single farm although it was not possible to establish if they shared a related parentage.

Any attempt to determine the earliest point at which preinfection produces resistance to experimental infection was limited by the fact that the recognition of preinfection was based on the presence of two cyst populations differing in size and degree of larval development. Since there was some variation in any single population, it was difficult to diagnose preinfection at autopsy in calves experimentally infected under 9 days of age and killed 14 days later. Over this age the rapid growth of the naturally acquired cysticerci during their 3d week of development (McIntosh and Miller, 1960) enabled these to be distinguished from the experimental cysts of 14 days duration. With this in mind the resistance of the normal and preinfected calves infected from 9 to 20 days after birth were compared. Six of 10 preinfected calves were resistant compared to 3 of 10 normal calves. In older calves aged 21 to 40 days, 9 of 15 preinfected calves were resistant compared to 2 out of 10 normal calves.

FIGURE 1. Portion of heart muscle from calf infected 14 days previously. Numerous small cysts together with single cyst from previous natural infection. ×1.5.

The Relationship between the Ingestion of Colostrum from Immune Dams and Subsequent Resistance to Experimental Infection

Since adult East African cattle uniformly show a marked degree of resistance to experimental challenge with *T. saginata* eggs, and since this is based partly at least on immunity acquired from previous exposure (see discussion), it was thought possible that calves might acquire a degree of immunity from the ingestion of colostrum. This has been shown to occur in a variety of viral, bacterial, and protozoal diseases of bovines (Brown, 1958; McDiarmid, 1946; Kerr and Robertson, 1946). Accordingly, 22 of the 66 calves were removed from their mothers and reared without access to colostrum. A comparison of the results of experimental infection of the two groups showed that 8 of the 22 (36 percent) colostrum-free calves and 19 of the 44 (43 percent) control calves were resistant. Thus ingestion of colostrum did not confer any significant protection against cysticercosis. Moreover, the majority of the resistant calves were also preinfected calves.

3. EXPERIMENTAL CYSTICERCOSIS IN CALVES AGED 6 TO 20 WEEKS

The work reported here deals with a group of calves reared for a longer period in a cysticercosis-free environment from 24 hours after birth. The results are discussed with particular reference to acquired immunity and age immunity.

East African calves: Each of 21 calves aged from 46 to 140 days and each of 2 calves aged 2 days received 15,000 *T. saginata* eggs orally. The autopsy results showed that of the 23 calves only the two 2-day old calves were considered by our criteria to have been suscept-

ible to infection.

Seventeen of the 21 older calves were shown to have been preinfected at autopsy; i.e., each was infected with cysticerci acquired previously to the experimental infection. All of these cysticerci were mature specimens except that they were somewhat smaller than are usually encountered at meat inspection. The average number per calf was 10.8 ± 16.3. Small numbers of very immature cysticerci (21.6 ± 17.2) which had developed from the 14-day experimental infection were found in 5 of the 17 preinfected calves.

Two of the four calves which were not preinfected were lightly infected with 24 and 40 cysticerci respectively from the experimental infection.

Scottish calves: Each of seven calves which received 15,000 *T. saginata* eggs orally was killed 28 days after infection. The autopsy results showed that all seven calves were markedly susceptible to infection. Cysts from a previous infection were not found.

4. EXPERIMENTAL CYSTICERCOSIS IN CATTLE AGED 3 MONTHS TO AGED

In the experiments described here the susceptibility of older calves and adult cattle to experimental infection was studied. The experiments differed from those described previously in that all of the cattle were reared and maintained under normal commercial conditions.

East Africa

Calves. Twenty-one calves aged 3 to 13 months and reared under range conditions were each infected with *T. saginata* eggs; the dose ranged from 2 to 30 thousand eggs. When autopsied 13 to 19 days later small numbers of mature cysticerci from previous natural infection were found in 17 of the 21 calves. The cysticerci were both living and degenerate. Eight of the calves had living cysts only, five both living and dead cysts, and four dead cysts only. All of these calves were resistant to the experimental infection in that 12 were completely immune, 3 showed a total of 7 normal 14-day cysts, and 2 a total of four small green lesions. Each of the latter was found in skeletal muscle and was about 2 to 3 mm in length; the green color at the periphery of the lesion gradually faded into the muscle. Histological sections showed each to be composed of a central necrotic area in which the only recognizable elements were dead eosinophils. This area was surrounded by an inner thin ring of mononuclear cells and an outer dense inflammatory infiltrate largely composed of eosinophils. Muscle fibres in the infiltrate were widely separated and on cross-section were homogeneous and frequently basophilic. The outer boundary of the infiltrate was not sharply demarcated, but extended irregularly between normal muscle fibres.

Four calves in which mature cysts were not detected were also resistant. Three were completely resistant while the fourth had five green lesions similar to those noted above.

Adults: Forty-eight commercially-reared cattle received *T. saginata* eggs and were autopsied 14 to 44 days later. Thirty of the 48 were found to be infected with mature cysts acquired before the experimental infection. Nine cattle had living cysticerci, 11 both living and dead cysticerci, and 10 dead cysticerci only. The number of living cysts in each carcass ranged from 0 to 72, averaging 7.9 ± 17.0. The number of dead cysts per carcass ranged from 0 to 10, averaging 0.9 ± 1.7. All 48 cattle were completely resistant to challenge. Three showed 1, 3, and 6 small green lesions respectively.

Twenty-two cattle which had not been experimentally infected were autopsied at the same time. Mature cysticerci were found in 16 of these. In nine the cysticerci were living, in four both living and dead cysticerci were present, and in three cattle the cysticerci were dead. The number of living cysts in each carcass ranged from 0 to 30; average 5.2 ± 7.7. The number of dead cysts per carcass ranged from 0 to 12; average, 0.9 ± 2.6. It is apparent that within the conditions of the experiment the challenge of infected cattle did not significantly alter the numbers or viability of established cysticerci.

Scotland. Five bovines each received 40,000 eggs and were autopsied 20 to 32 days later. The two youngest aged 3 and 12 months, respectively, were considered to be susceptible by the criteria adopted. By the same standards 2 were moderately resistant and one highly resistant.

5. THE NATURAL INCIDENCE OF CYSTICERCOSIS AND THE IMMUNE STATUS OF THE HOST IN SAMPLES OF EAST AFRICAN CALVES AND CATTLE.

All of the work described in the preceding sections was carried out with calves and cattle born on the laboratory farm or obtained from farms within a hundred miles of the laboratory.

Since East Africa is over 600,000 sq mi in area and, since there is a great range of stock-rearing practices, efforts were made to determine if the results previously obtained were typical of the territory as a whole. This was done by infecting experimentally small groups of calves or cattle in representative beef-rearing farms. These were autopsied approximately 14 days later so that both the success of the experimental infection and the degree of natural infection acquired previously could be determined.

The numbers of calves, their ages, and the incidence of naturally acquired cysticercosis is shown in Table III. The cysticerci in the group

TABLE III. *Incidence of naturally acquired cysticercosis in calves born on farms in Kenya and Uganda.*

Age in months	0.5	1	2	3 to 4	5 to 9
Number of calves infected	$\frac{0}{9}$	$\frac{6}{14}$	$\frac{12}{15}$	$\frac{12}{21}$	$\frac{13}{21}$

aged 1 month were all immature and had been acquired at least 12 days previously; those from the 2 to 3 month old calves were both immature and apparently mature, although small. The cysticerci from the older age group were all mature or degenerate.

Forty-two of the calves had each received 10,000 *T. saginata* eggs 14 days previously. The autopsy results showed that 5 of the 14 calves in which evidence of previous natural infection was not found were susceptible to the experimental infection; all were under 3 months of age. Of the remainder, six were completely resistant and three almost completely so. Only 1 of the 28 preinfected calves was susceptible; it was 2 months old. Of the remaining 27 calves, 13 were completely resistant and 14 became very lightly infected.

DISCUSSION

The results described in the first section are concerned with the occurrence of cysticercosis in four groups of young calves reared in the following ways: (1) Hand-reared from within a day or so of birth under cysticercosis-free conditions; (2) hand-reared on their farms of origin under normal management conditions; (3) reared out-of-doors by dams which had received *T. saginata* eggs during the second half of pregnancy; calves unhandled by man; (4) reared out-of-doors by their dams and unhandled by man.

In the first group cysticercosis was not found in any of the 11 calves autopsied between 3 and 15 days of age, but did occur in 18 of the 22 calves autopsied when between 16 and 55 days of age.

That the source of infection was the farm of origin of the calf rather than our laboratory calf pens is indicated in three ways. First, the relative sizes and degrees of development of the cysticerci in each of the infected calves were compatible with an infection acquired about the day of birth. (McIntosh and Millar, 1960; Urquhart, in preparation). Secondly, cysticercosis was found at autopsy in 4 of the 8 calves in group 2. Thirdly, the precautions observed in our calf pens made these an unlikely source of infection.

The inference that these calves were infected on their farms of origin and, therefore, before they were more than a day or so old suggested that prenatal infection had occurred in a number of calves at least. Evidence for this was not found in 17 of the 18 infected calves of group 1 in which the sizes of all of the cysts were compatible with postnatal infection. The single exception, a calf killed at 17 days of age, carried a cyst whose 9 mm host-capsule surrounded a cysticercus of 0.75 mm diameter. A cyst of this type would be associated normally in our experience with a minimal infection period of 25 days, and may, therefore, have originated in a prenatal infection.

Our inability to find cysts in any of the 11 hand-reared experimental calves autopsied under 15 days of age may also be cited in support of the theory that the high incidence of cysticercosis in our hand-reared calves was due to infection acquired during the short period of postnatal life on the farms of origin. Had prenantal infection commonly occurred one might reasonably have expected to find grossly visible cysts before 12 or 13 days of age, the minimal period for cysts of postnatal origin to become apparent.

Further evidence that prenatal infection does not commonly occur is, first, the absence of cysticercosis in the 10 unhandled calves of group 4 which were suckled with their dams until 13 to 24 days of age and, secondly, our inability to produce prenatal cysticercosis in calves (group 3) by the administration of *T. saginata* eggs to pregnant cows.

The combined results of the experiments described above indicate that while prenatal cysticercosis infection may occur, it is probably uncommon. This is supported by the work of Ginsberg (1958) and McManus (1960). The former showed that during 1957, 4.5 percent of calves 2 to 14 days old slaughtered at an abattoir near Nairobi were found to be infected with cysticercosis at meat inspection. If cysts in calves of under 10 days of age are accepted as cases of prenatal infection, it is apparent that the phenomenon occurred in a small percentage of calves only. More recently McManus working in the same abbatoir showed that during 1957 and 1958, only 3.07 percent of 14,855 calves had cysticercosis; approximately 80 percent of all the calves examined were under 21 days of age.

The high incidence of immediate postnatal infection in our calves drawn from six separate farms suggests that the incidence of taeniasis must be very high among African stockmen. That this is so is shown by Fendall (1959) who reported that in 1958 a tapeworm eradication campaign showed that 30 percent of 2,715 Kenya agricultural workers were infected. In addition to the high rate of infection in the population, a second important factor is the number of *T. saginata* eggs passed daily by an infected person. Penfold, Penfold and Phillips, (1937) concluded that an average of 8 gravid segments per day are passed by an infected person, while Silverman (1954) found that the number ranged from 0 to 12 per day; each segment contains approximately 100,000 eggs (Monnig, 1956). Our experience has confirmed that of Alcaraz (1932), Pardira (1932), and Frangani (1933) who concluded that the number of eggs in the feces was as important as the number of eggs in each segment, since we found the following numbers of *T. saginata* eggs per gram of feces in a series of dispensary patients: 120, 2700, 3000, 4200, 7200, and 33000. If these numbers of eggs per gram are typical it is probable that taenia eggs are usually present, though in small numbers on the hands and under fingernails of infected persons. If, therefore, the mouth of a calf should be handled shortly after birth, to clean mucus out of the mouth or to force it to drink from a pail, for example, it is likely that the animal would become infected.

Since it was not always possible to enucleate a cysticercus by gross dissection from every cyst of 5 to 10 mm diameter or to find consistently a cysticercus in histological sections of individual lesions, it is probable that a proportion of cysticerci degenerate during the developmental stages in the tissues. This phenomenon was observed by Penfold, Penfold and Phillips (1936) in experimental *C. bovis* infections in adult cattle, and by Lucker and Douvres (1960) in an experimentally infected calf; the former considered that the isolation of one viable cysticercus from 100 lesions was quite consistent with an infection of 11 weeks duration. Degeneration of developing cysticerci was also observed by Leonard and Leonard (1941) in *C. pisiformis* infections in rabbits.

The conclusion that dairy calves in East Africa commonly become infected with cysticercosis the first day or so of postnatal life was confirmed in the experiments described in the second section designed to study the results of experimental infection of young calves. Twenty-seven out of 66 calves showed cysts at autopsy which, judging by size and degree of development, indicated infection acquired at or about the time of birth. The size and degree of development of the naturally acquired cysts in all but one of the 27 preinfected calves were compatible with infection acquired the first day of life. The exception was a 14-day-old calf with two cysts whose degree of development suggested prenatal infection. This result tends to support the conclusion expressed previously that prenatal infection is possible but relatively uncommon.

The high degree of resistance to experimental infection shown by calves naturally infected on the first day of life is of particular interest in two ways. First, despite the fact that these natural infections were almost certainly light (the numbers of cysts in each calf ranged from 1 to 60) a marked degree of immunity was produced to a relatively massive challenge at least several hundred times the size of the initial infection. Secondly, this high level of resistance was demonstrable as soon as 9 to 40 days after the primary infection. A comparable result was obtained by Weinmann (1958) who showed that mice lightly infected with cysticercoids of *Hymenolepis nana* were markedly, if not absolutely, immune to heavy challenge 3 to 6 days later.

These results provide a probable explanation

for the results reported by Duthie and Van Someren (1948) in Kenya, who were unable to produce heavy experimental infections of cysticercosis in Kenya calves despite the administration of many thousands of *T. saginata* eggs; e.g., three calves given 44,000, 39,000, and 240,000 eggs each were shown at autopsy to possess only 17, 1, and 0 cysts, respectively. It is likely that the calves became infected shortly after birth and had developed acquired immunity before experimental infection. The authors stressed the difficulty of rearing calves under cysticercosis-free conditions.

The finding that approximately a quarter of the normal calves were also resistant to infection indicates that the innate resistance of the individual plays a considerable role in determining the size of the infection. This statement must be qualified by the possibility that the resistance of at least some of these calves was due to one of two other factors: First, the presence of cysts acquired from an earlier infection and undetected despite a careful autopsy; second, acquired immunity stimulated by a light natural infection which had been acquired in early calfhood and subsequently degenerated and disappeared.

The histopathology of the circumscribed lesions associated with cysticerci developing from experimental infections will be described elsewhere. Nevertheless, it is relevant here to note that viable cysticerci may or may not be present in the lesions. Also, as noted previously it was not possible always to enucleate a cysticercus by gross dissection from the large cysts acquired by natural infection.

In the experiment described in the third section in which 21 calves of 6 to 20 weeks were experimentally infected, 17 were shown to have been naturally infected previous to experimental challenge. Since the calves were reared from after the first day of life under conditions designed to prevent accidental infection, it is reasonable to conclude that they also were infected on the first day of life. Had this not been so one might have expected to find cysticerci whose degree of development was intermediate between mature and 14 days duration.

Of the 17 preinfected calves, 12 were completely resistant to experimental challenge and 5 were almost completely resistant, the numbers of young cysticerci ranging from 3 to 42 per calf. Two of the four normal calves were completely insusceptible to infection and two were very lightly infected with 24 and 40 cysticerci, respectively, in each carcass.

Our inability to produce infections proportional to the number of eggs which these calves received was not due to poor egg viability as the two 2-day-old calves were found to be heavily infected at autopsy. Nor did it appear to be caused by age immunity in the calves, since the seven 3-month old Glasgow calves were also heavily infected. A third possibility is breed resistance. Many European breeds of cattle in East Africa have a native Boran ancestry which could conceivably confer gneetic resistance to cysticercosis. This is unlikely, however, to be a significant factor, since several Boran calves were among those heavily infected in the experiments described in the second section and in the field experiments.

We consider that the resistance of the 17 preinfected calves was due to immunity stimulated by natural infections acquired in early postnatal life. The resistance of the four nonpreinfected calves may be attributed to one of the two following factors: Acquired immunity stimulated by the presence of undetected mature cysts or by an early natural infection which had subsequently degenerated and disappeared; or, natural, resistance, since we have previously indicated that a proportion of East African calves exhibited it.

Of the 69 East African calves and cattle reared under commercial conditions and challenged at the laboratory (section 4), each of 66 was completely resistant to the experimental dose of *T. saginata* eggs. In six of these a small number of macroscopic lesions each composed of inflammatory cells around necrotic material were found and were possibly associated with the death of cysticerci developing from the experimental infection. In the remaining three, calves 2, 3, and 8 months old, only 1, 5 and 1 developing cysts, respectively, were found which appeared macroscopically normal. A similar result was reported recently by Froyd and Round (1960) in Kenya, who failed to infect any of 14 adult cattle with doses of *T. saginata* eggs ranging from 1,000 to 170,000. At autopsy, 12 of these cattle were shown to have been naturally infected previously.

This remarkable degree of resistance to eggs of proven infectivity to young calves is probably attributable to age per se or to acquired immu-

nity from previous infection. Age immunity was not encountered by Penfold, Penfold and Phillips (1936) in Australia who produced generalized cysticercosis in all of 88 adult steers previously unexposed to the disease. In the present experiment only one of four Scottish cattle over 1 year old was shown by us to be markedly susceptible to infection; the remaining three were lightly infected. It is highly improbable that these cattle had any previous contact with cysticercosis as the disease is uncommon in Scotland (Silverman, 1954). Nevertheless, since all of the Australian and Scottish cattle were susceptible in some degree to the experimental infection, and since all of the East African cattle over 8 months of age were completely insusceptible, it is likely that acquired immunity rather than age immunity was primarily responsible for the resistance of the latter.

Previously, we have shown that naturally acquired cysticercosis in calves stimulated resistance to subsequent experimental challenge. Also, Penfold et al (1937) have shown, first, that each of 7 experimentally infected adult cattle were completely immune to reinfection some months later and, secondly, that each of 20 cattle which had grazed for 4 years on pasture treated with sewage was completely insusceptible to a challenge of 400,000 *Taenia saginata* eggs. We consider it likely, therefore, that the resistance of the majority or all of the East African cattle was acquired through previous exposure. Evidence of previous infection in the form of living or dead mature cysts was found in 17 of the 21 calves and 46 of 70 adult cattle. Furthermore, our inability to find cysts in the remainder cannot be regarded as indicating previous freedom from natural infection for two reasons: First, the possibility of undetected cysts in the carcass; second, the cysts of an earlier infection may have degenerated and subsequently disappeared, (Penfold et al, 1937; Lucker & Douvres, 1960) and the host remained immune to further infection.

From the results reported here it is apparent that successful experimental infections of cysticercosis in East African cattle have been produced in calves up to 80 days only. Thereafter, calves and cattle were for all practical purposes, completely insusceptible to experimental infection. The two factors considered primarily responsible for this degree of resistance are previous infection and, to a lesser extent, innate resistance of individuals. The calves used in the preceding experiments were mostly of dairy origin in which natural infectious were frequently acquired about the first day of life. Since the cattle in this experiment were reared commercially, it is not possible to say with certainty at what age the primary infection were acquired. Nevertheless the results of the survey on beef-ranches and African farms (section 5) have indicated that the incidence of cysticercosis and the early acquisition of resistance found in calves from the farming area around Nairobi is probably typical of the beef-rearing areas of East Africa except in one respect: Beef calves (apparently) do not commonly become infected until the first or second month of life. This difference in age incidence is probably due to the degree of handling experienced by dairy calves compared to calves born on beef ranches.

It appears, therefore, that in East Africa susceptible calves are usually infected in the first few weeks of life and subsequently are completely immune to reinfection within a few weeks. This immunity is life-long although the cysts derived from the initial infection may degenerate or disappear. In a proportion of cattle the cysts survive during the entire life of the host.

The conclusion that *C. bovis* may live in the tissues of the host for several years is contrary to Penfold et al (1937), who during serial autopsies of 88 adult cattle infected experimentally with *T. saginata* eggs, found no viable cysts in infections of over 9 months duration.

The three most probable explanations for this apparent difference in larval longevity observed in East Africa and in Australia are:

1) the age of the host when first exposed to *C. bovis* infection may determine the longevity of the infection. In the Australian experiments the cattle were adult when experimentally infected; in East Africa it appears that the majority of cattle are first exposed as calves.

2) the size of the initial infection may determine the immune response of the host and thus the larval longevity. In the Australian experiments each animal received aout 400,000 eggs; in East Africa naturally acquired calfhood infections are relatively light.

3) the Australian and East African strains of *C. bovis* may be biologically different, the former possessing greater longevity.

Apart from the three hypotheses outlined

above to explain the apparent longevity of *C. bovis* in East African cattle, two further possibilities were envisaged. The first depended on the work of Leonard and Leonard (1941) who showed that, while passively immunized rabbits were resistant to oral infection with eggs of *T. pisiformis,* they were completely susceptible to the intravenous administration of hatched oncospheres; they concluded that these results indicated that the intestine played a vital role in the mechanism of acquired immunity. I had considered it possible that East African cattle infected in calfhood and subsequently immune to oral infection might be infected parenterally by biting flies which had previously fed on feces or other material containing *T. saginata* eggs; by by-passing the intestine it might be possible to repeatedly re-infect cattle with *C. bovis* despite a solid immunity to infection via the oral route. Experiments to test this hypothesis will be described in another paper; it was concluded that this method of infection in adult cattle was very improbable.

The second possibility was that the immediate postmigratory stages of *C. bovis* might remain undetected in the body for long periods and only resume development on the death of established mature cysts; this dormant phase is known to occur in certain nematode infections (*see* Soulsby, 1957). I consider that this is unlikely, since immature cysts were not observed in the muscles or organs of approximately 150 adult bovines autopsied by me for cysticercosis over a period of 3 years.

The 70 adult cattle autopsied in this experiment showed an overall incidence of cysticercosis of 66 percent. During the same period, 340 cattle of similar origins and also maintained at the laboratory grazing area were sent from the laboratory to the Nairobi abattoir. The incidence of cysticercosis in these cattle as revealed by routine meat inspection incisions carried out by the technique described by Ginsberg (1956) was 41 percent. These results are significant (chi-square = 13.47 $P < 0.01, > 0.001$) and suggest that of all carcasses subjected to extensive dissection and shown to be infected with cysticercosis about two-thirds are detected by competent meat inspection techniques alone.

The results reported in this paper have considerable significance in any scheme of prevention or control of cysticercosis. It is apparent, for instance, that the conditions which Penfold et al (1936) envisaged as desirable for the eradication of cysticercosis in enzootic areas, namely the deliberate infection of young calves, occurs naturally in East Africa; despite this, the incidence of cysticercosis at meat inspection is very high (Ginsberg et al, 1956). Penfold et al had assumed that the cysts produced deliberately would degenerate within a few months and that the hosts would remain immune to further infection, until slaughtered. In East Africa natural infection in early life followed by absolute immunity to reinfection appears to be general. Unfortunately, a proportion of cysts from these early infections appears to survive in the host tissues for years.

A modified system of control commonly practised by ranchers in East Africa and also presumably based on Penfold's conclusions on cyst longevity (1937) consists of grazing cattle away from native dwellings during the last year of the animals' lives. From our work, this method of control would appear to be of no value.

The immunity of mature cattle to reinfection indicates, first that a cysticercicidal drug could be used at any convenient point in the animals' lives, and that there is no necessity to use the drug immediately before slaughter with possible consequences to the palatability or safety of the meat for human consumption. Second, it is possible that vaccination with, for example, x-irradiated eggs, on similar lines to the technique successfully employed for *Dictyocaulus viviparus* in calves (Jarrett et al, 1959) might be of value in the eradication of the disease.

SUMMARY

1. Thirty-three calves born on dairy farms in East Africa were brought within 24 hours of birth to the laboratory where they were hand-reared under conditions designed to prevent cysticercosis infection.

The calves were killed and autopsied from 3 to 55 days of age. Eighteen out of 22 calves over 16 days of age were shown to be infected with cysticercosis.

Examination of the lesions and parasites from all of the infected calves indicated that with the possible exception of one calf the disease had been acquired on or about the day of birth. The exception was infected with a cysticercus whose size and degree of development suggested a prenatal origin.

Attempts to produce prenatal infection of

calves by the oral or intravenous administration of *T. saginata* eggs to each of 8 pregnant cows were unsuccessful.

Ten calves born on the laboratory farms, unhandled by man and suckled by their dams for 10 to 29 days, were shown at autopsy not to be infected with cysticercosis.

The results indicate that in one area of East Africa calves of dairy origin are commonly infected with cysticercosis in the first day or so of life, and prenatally acquired cysticercosis is a relatively infrequent phenomenon.

2. Each of 66 Kenya calves aged from 1 to 40 days and reared from within a day of birth under conditions designed to prevent accidental infection with bovine cysticercosis was experimentally infected with 10,000 to 20,000 *T. saginata* eggs.

At autopsy 27 of the calves were found to be relatively resistant to the experimental infection. This appeared to be due to two factors:

(i) acquired immunity due to natural infection previous to the experimental infection. These natural infections were characterized by very small numbers of cysts whose size and degree of development indicated infection on or about the day of birth. In one calf prenatal infection was suspected.

(ii) innate individual resistance.

The presence of colostrum in the postnatal diet of calves from immune dams does not appear to confer any significant immunity to cysticercosis, since 22 of the calves reared without colostrum were not more susceptible to experimental infection than the others.

3. Each of 21 Kenya calves aged 46 to 140 days and reared from within a day of birth under conditions designed to prevent accidental infection with bovine cysticercosis were experimentally infected with 15,000 *T. saginata* eggs.

At autopsy 17 of the calves were infected with mature cysts from natural infections apparently acquired on the first day of life. Very small numbers of immature cysts from the experimental infection were found in 5 of these calves. Of the 4 calves not infected with mature cysts, two were completely resistant to the experimental infection and two were lightly infected.

The resistance of the previously infected calves to experimental infection was considered to be due to acquired immunity and that of the normal calves to innate resistance or to acquired immunity stimulated by the presence of undetected cysts.

Age immunity was shown not to be a significant factor

4. In East Africa, single doses of *T. saginata* eggs ranging from 500 to 150,000 were administered orally or intra-ruminally to 48 adult cattle and 21 calves, aged 3 to 14 months, reared and maintained under commercial stock-rearing conditions. In Scotland, each of five cattle aged 3 months to adult received a single dose of 42,000 eggs.

At autopsy 13 to 19 days later, 18 of the 21 East African calves were found to be completely resistant to the experimental infection; in the remaining 3 a total of seven developing *C. bovis* cysts was found. Seventeen of the calves were infected with living, living and dead, or dead mature cysts acquired from a previous natural infection. At autopsy 15 to 45 days later, all 48 adult cattle were found to be completely resistant to experimental infection. Thirty of the cattle were infected with living, living and dead, or dead mature cysts acquired previously. In the Scottish cattle, experimental infections ranging from one to many hundreds of *C. bovis* cysts were established; none showed evidence of previous infection.

The results are discussed in relation to other work on age immunity and acquired immunity, and it was concluded that in East Africa susceptible calves are usually infected in the first few weeks of life and are subsequently completely immune to reinfection within a few weeks. Immunity is apparently life-long although the cysts derived from the initial infection may degenerate or disappear. In a proportion of cattle the cysts survive during the entire life of the host. The conclusion that *C. bovis* may live for several years in the host's tissues is contrary to previously reported work; a number of explanations are offered to explain this apparent contradiction.

Finally, the significance of the results in the control of cysticercosis is discussed.

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THE MAINTENANCE OF *ECHINOCOCCUS MULTILOCULARIS* IN GERBILS *(MERIONES UNGUICULATUS)* BY INTRAPERITONEAL INOCULATION

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Infections of *Echinococcus multilocularis* (Em) have been produced by feeding eggs to several strains and species of laboratory rodents (Rausch, 1954; Mankau, 1956, 1957; Vogel and Schumacher, 1957; Sadun et al, 1957; Yamashita et al, 1958 Lubinsky, 1960a, b). Sadun et al (1957), in our laboratory, reported that the CFW strain of albino mouse fed eggs of Em was resistant to infection, but that the cotton rat, *Sigmodon hespidus,* was very susceptible. Infection in cotton rats develops rapidly (in 1 to 2 months) and eventually kills the host. Cysts grow to 1 cm in diameter and emanate first from liver and lung tissue, and finally invade other viscera until the organs are compressed or displaced. Host and parasite tissue are so intimately mingled that complete separation is impossible.

In continuing our search for a practical method of obtaining Em cyst material as free from host tissue as possible and also for a practical technique of maintaining the parasite in the laboratory without the use of eggs of Em, experiments were initiated to produce infections by means of intraperitoneal inoculation of viable cyst cells from animal to animal. The transplantation of *Echinococcus granulosis* (Eg) and Em cyst tissue via intraperitoneal inoculation has recently been reported by Yamashita, Ohbayashi and Konno (1957); Schwabe, Schinozi and Kilejian (1959), and Lubinsky (1960a, b). Continued attempts to render our CFW mice susceptible to infection by treatment with cortisone either before or simultaneously with oral feeding of eggs resulted in production of only very limited parasitic material; however, feeding of eggs to gerbils resulted in fairly good infections, suggesting that these rodents might be suitable animals for further investigation. Using infected gerbils and cotton rats as the sources of viable cyst tissue, intraperitoneal infections in gerbils (with occasional passage through cotton rats) have been established and maintained through nine serial transfers in our laboratory continuously from April 1958 to April 1961. Our experience with this technique is outlined.

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MATERIALS AND METHODS

Host animals: Ten albino rats, 75 mice of CFW, C_3H, dba, C_{57}, and A strains, 17 hamsters, 13 guinea pigs, 7 rabbits, 25 gerbils and 10 cotton rats were used in the experiment to test for susceptibility to infection by intraperitoneal injection. For maintenance, 14 cotton rats and 73 gerbils were inoculated. Young animals were preferred to adults, but both were tested.

Inoculum: E. multilocularis cysts growing in cotton rats or gerbils were partially freed from host tissue and homogenized 15 to 20 seconds in a generous amount of physiological saline in the Waring Blendor. To insure the removal of soluble substances which were very toxic to young hamsters and gerbils, the suspension was centrifuged at low speed or allowed to settle by gravity, and the supernatant was discarded. Cyst tissue was resuspended in fresh saline so that the inoculum was as thick as could be easily handled with a syringe and 22-gauge needle. Two thousand units of penicillin G and 2000 micrograms of streptomycin sulfate were added per ml. When some animals of species other than gerbils or cotton rats were inoculated, 1 to 2 ml of normal serum from the donor species were added to each 10 ml of inoculum.

Dosage and inoculation: Small animals (under 30 gms) were injected intraperitoneally with about 0.2 ml of the cyst suspension. Larger animals received 0.4 to 1 ml, and the rabbits were given 5 ml each. To insure viability of the cells, inoculations were made as quickly as possible, usually within the hour, after removal of the cysts from the donor.

For maintenance of *E. multilocularis* by intraperitoneal passage from gerbil to gerbil or gerbil to cotton rat, the inoculum was prepared as above and 0.2 to 0.4 ml of tissue suspension was injected intraperitoneally. Animals were autopsied 2 to 8 months later, and fresh tissue suspension subinoculated immediately. Several times when 1 or 2 gerbils in an experimental group were found to be negative or only very lightly infected, the remaining animals were reinoculated with fresh cyst material. To date nine transfers have been made into a total of 66 animals over a 3-year period (table I).

TABLE I. *Intraperitoneal passage of* echinococcus multilocularis *cyst material over a 3-year period.*

Transfer Experiment	Recipient	Date	Number	Age	Month autopsied	Results
1	Gerbil	4/25/58	5	young	2	All heavily infected Transferred to gerbils-2.
2	Gerbil	6/13/58	2	adult	5	One heavily infected Transferred to gerbils-3.
3	Gerbil	11/13/58	2	young	6	Both animals heavily infected. Transferred to gerbils-4.
4	Gerbil	4/17/59	4	young	3	One of four moderately infected. Transferred to gerbils 5–1 and cotton rats 5–2.
5–1	Gerbil	7/23/59	7	4 young 3 old	3	One heavily infected. Transferred to gerbils 6–1.
5–2	Cotton rat	7/23/59	7	young	4	Three slightly infected. Transferred to cotton rats 6–2.
6–1	Gerbil	10/28/59	5	2 old 3 adult	6	Three of four positive. Transferred to gerbils 7–1.
6–2	Cotton rat	12/10/59	5	adult	5	Three positive. Transferred to gerbils 7–2 and cotton rats 7–3.
7–1	Gerbil	5/18/60	7	4 adult 3 young	7	One heavily infected. Transferred to gerbils 8–1.
7–2	Gerbil	5/18/60	8	young	7	One of four positive. Transferred to gerbil 8–2.
7–3	Cotton rat	5/18/60	2	young	8	One heavily infected. Transferred to gerbils 9.
8–1	Gerbil	1/13/61	7	young		All died in shock
8–2	Gerbil	2/22/61	6	young		One infection palpable. Reinoculated 5/26/61
9	Gerbil	3/ 3/61	6	young		All show evidence of infection by palpation.

RESULTS

Infections with *E. multilocularis* produced by intraperitoneal inoculation of 75 mice were light. Four of five adult CFW mice inoculated with cyst material from an infected cotton rat developed a few small cysts after 3 months of infection (fig. 1). These cysts contained scolices

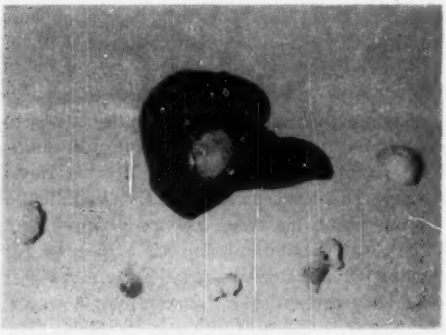

FIGURE 1. Cysts of *Echinococcus multilocularis* in CFW white mice 3 months after intraperitoneal inoculation of cyst material.

which were viable since they produced moderately heavy infections when transferred to three young gerbils. Twenty weanling CFW mice injected with 0.1 ml of inoculum from an infected gerbil showed single cysts in 3 animals autopsied after 5 months and two others showed caseous degenerated cysts. However 6 C_3H and 3 dba weanling mice, and 14 A strain, and 10 CFW adults inoculated at the same time were not infected. Three of 23 mice not given gerbil serum in the inoculum developed cysts as compared to 2 of 20 mice which received the serum mixed inoculum. In general, adult mice were refractive to infection.

Hamsters were particularly sensitive to the inoculum. In one experimental group six animals died in shock shortly after injection of the cyst suspension. When the inoculum was carefully washed, injected animals survived, but no cysts were found in nine hamsters autopsied. The 7 rabbits and 11 guinea pigs examined after 3 to 5 months also showed no evidence of infection. One small cyst was found in 1 of 6 weanling albino rats autopsied after 3 months. On the other hand, 10 cotton rats inoculated with material from either cotton rats or gerbils (no serum) were all well infected in 4 to 6 months, and some died apparently from the infection.

There was variation in the infections which developed in the gerbils (fig. 2). Thirteen lots of gerbils, totaling 67 animals, were given intraperitoneal injections. Thirty-three animals were

FIGURE 2. Experimental infection with *Echinococcus multilocularis* in the gerbil (*Meriones unguiculatus*).

autopsied; of these 23 (70 percent) were infected. To produce infections with cysts large enough to detect by palpation of the abdomen, reinoculation was necessary with some lots of gerbils. Others were as heavily infected as cotton rats after a single injection. It has been possible in spite of this variability to maintain the infection in the laboratory.

The data in table I outline our experience on the serial passage of *E. multilocularis*. On the eighth passage we lost our gerbil to gerbil strain because all the young gerbils died in shock. Fortunately, on the fifth passage we had made a gerbil to cotton rat transfer, carrying it in cotton rats (6-2) and passing it to gerbils and cotton rats on the seventh transfer. We now have a gerbil to gerbil passaged infection and a cotton rat to gerbil transfer.

Cotton rats are more susceptible than gerbils. We lost many cotton rats presumably from infection, and all survivors except in one instance (5-2) were heavily infected. The infection in the cotton rat differs in some degree from infection in gerbils. In the cotton rat cysts grow to 1 cm or slightly larger and contain hydatid fluid. In the gerbil, cysts which develop in the peritoneal cavity are cauliflower-like (fig. 3) resembling

FIGURE 3. Cyst masses of *E. multilocularis* in the peritoneal cavity of gerbils.

the type of infection reported by Rausch and Schiller (1951) in the natural microtine host in Alaska.

DISCUSSION

The purpose of this research project was to explore the possibility of maintaining *E. multilocularis* in the laboratory by intraperitoneal passage. This has been successfully accomplished for the past three years. The gerbil was found to be a susceptible host and has been used for this purpose. Cotton rats are the preferred host since they are extremely susceptible; however, they are very difficult to keep in the laboratory, are very feral, cannot be purchased commercially; and in our limited experience, they are difficult to breed. The docile gerbil is an ideal laboratory animal and can be purchased commercially.

In the gerbil infected by intraperitoneal inoculation, the cyst material in the peritoneal

cavity is frequently free or only superficially attached by connective tissue and can be separated from host tissue with no difficulty. The liver is often involved, and the liver cysts resemble those produced by feeding ova; i.e., they are usually larger and contain sufficient fluid to aspirate. Many scolices almost entirely free of other membranes can be collected from lightly homogenized cyst material.

Lubinsky (1960a, b) reported intraperitoneal passage of *E. multilocularis* from cotton rat to cotton rat, and on the third transfer passaged his material into gerbils and white mice. He studied the initial stages of the infection intensively and found that with each transfer an increased growth rate of the cyst material was observed. Yamashita et al (1958) fed 2-month-old gerbils with eggs of *E. multilocularis* and found this host species to be very susceptible to infection.

We were interested in the maintenance of the strain and susceptibility of various animal species for this purpose, and did not autopsy animals during the period immediately following injection of cyst material. Some of the species tested may well have harbored transient patent infections, but at autopsy, 2 to 8 months after passage, were negative. Approximately 50 percent of our gerbils and cotton rats died in the interim period between injection of cyst material and autopsy. No attempt was made to ascertain infection or cause of death in these animals.

Our experience indicates that mice (CFW, dba, C_3H, C_{57} and A) strains are refractive hosts for *E. multilocularis* by intraperitoneal passage or by feeding viable eggs. Experiments with 82 mice, ranging in weight from 10 to 31 g, in some instances injected with cortisone, which were fed eggs of Em, resulted in only three infected animals (two receiving cortisone). Rausch (1954) and Mankau (1956, 1957) studied the histogenesis of *E. multilocularis* in the liver of rodents fed eggs of this parasite. Vogel and Schumacher (1957) reported that in 188 mice fed eggs of *E. multilocularis* most became infected and showed cysts in their livers. Mankau (1956) reported 70 percent of the cysts in mice infected with *E. multilocularis* were fertile and 3 to 4 months of infection had to elapse for scolices to develop. Yamashita et al (1958) exposed many rodents to eggs of Em and found several, such as AKR, dba mice and species of *Clethrionomys, Microtus, Apodemus,* and *Meriones* to be good hosts. Scolices developed in 6 to 10 weeks. They reported CFW, C_3H, C_{57} mice and hamsters to be refractive hosts. Cysts, when found, were minute; scolices required as much as 5 months to form; and the tissue reaction was very severe.

Cameron (1960) recognized two subspecies of *E. multilocularis: E. m. multilocularis* which develops readily in the common red fox (*Vulpes vulpes*) of Europe and experimentally in white mice and *E. m. sibiricensis* which develops readily in the blue or arctic fox (*Alopex lagopus*) but with difficulty in some strains of white mice. Lubinsky (1960a, b) and Mankau (1956, 1957) have been able to infect white mice with material received from Alaska. In our work we have been unable to inject mice readily with *E. multilocularis* from the same source. It is evident that the intermediate rodent host plays an important role in the development of this parasite to the extent of influencing the number of rostellar hooks which develop on the scolex (Lubinsky, 1960b).

Yamashita, Ohbayashi and Konno (1957) produced secondary infection of *E. multilocularis* in mice by intraperitoneal injection using cyst material from the natural host, the red backed vole (*Clethrionomys rutilus*). They reported small cysts in their animals. Sadun et al (1957) studied experimental infections in cotton mice (*Peromyscus gorsyponus*), deer mice (*Peromyscus maniculatus*), field mice (*Peromyscus polionotus*), and white mice. After feeding eggs of *E. multilocularis* they found 4 of 14 cotton mice, 2 of 6 deer mice, and 4 of 6 field mice infected. Six white mice were refractive.

Schwabe, Schinozi and Kilejian (1959) reported age resistance to secondary *Echinococcus granulosus* infection in white mice. Mice 48 days or younger were highly susceptible to an intraperitoneal injection of cyst material; whereas mice 71 days or older were relatively resistant. The difference in susceptibility was related to the magnitude of the host cellular response. To obviate age and the cellular response associated with infection in rodents, we attempted to establish *E. multilocularis* in weanling and older CFW mice some of which were injected with cortisone at the time they were fed eggs. Our results indicate that a small percentage of weanling mice can be infected by feeding eggs or by

intraperitoneal passage of cyst material but the rate of infection is very low and the cysts are small.

In our attempt to enhance the survival of the infective inoculum (scolices and germinative membrane) in various experimental hosts, serum from the donor host was included in the inoculum. Our results show that this procedure did not improve the infectivity of the inoculum. Animals inoculated with and without serum showed similar results.

Our data on intraperitoneal injections of gerbils indicate that age perhaps may play a role in the degree of infectivity demonstrated by this animal species. Our inoculum of cyst material was not standardized but we observed that although older gerbils are susceptible to infection, young gerbils develop heavier infections.

SUMMARY

Echinococcus multilocularis has been maintained in the cyst stage in gerbils and cotton rats over a period of 3 years by means of nine serial transfers of viable cyst tissue suspensions inoculated into the peritoneum of the rodents. Masses of cysts containing motile scolices and relatively free of host tissue, are produced in 4 to 6 months. Mice and weanling rats are slightly susceptible to secondary echinococcus infection with *E. multilocularis,* but guinea pigs, rabbits, and hamsters are refractive to infection.

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ETHIOPOTAENIA TRACHYPHONOIDES GEN. N., SP. N. FROM THE CRESTED BARBET, *TRACHYPHONUS VAILLANTII* (RANZANI) (AVES), IN SOUTHERN RHODESIA

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In May 1959 a number of cestodes were recovered from the intestine of a Crested Barbet, *Trachyphonus vaillantii* (Ranzani), from the Fort Tuli region on the Southern Rhodesia-Bechuanaland border. On examination all the material except two specimens was found to be a hymenolepid species. The remaining two specimens represent a new genus in the sub-family DILEPIDINAE Fuhrmann, 1907 (DILEPIDIDAE Railliet and Henry, 1908).

Ethiopotaenia gen. n.

Diagnosis: Dilepidinae, with an unarmed scolex and no rostellum. Genital pores irregularly alternate; genital ducts pass between dorsal and ventral longitudinal excretory vessels. Female genitalia in anterior half of segment; testes usually in two lateral groups, sometimes joining across posterior margin of segment. Cirrus-sac extending beyond lateral excretory vessels; uterus thin-walled, sac-like, and later becoming lobed, but remaining between lateral excretory vessels.

Adults in Capitoniformes.

Ethiopotaenia trachyphonoides sp. n.

Description: All measurements in millimeters unless otherwise stated. Worms small in size: length 17 to 31; width 1.13 to 1.14 Scolex 0.53 in diameter, completely unarmed; rostellum absent. Suckers four in number, 0.17 to 0.18 in diameter. Neck 0.31 in diameter, 0.35 long. Mature and gravid segments wider than long. Typical mature segment 0.91 wide and 0.32 long. Typical gravid segment 1.12 wide and 0.54 long. Inner longitudinal muscles arranged in continuous circle, only interrupted by lateral genital ducts. Circle double; an outer layer of single fibers and an inner layer 2 to 3 fibers thick. Anlagen of testes appears about segment 40. Testes, 20 to 27 per segment, arranged in two lateral groups slightly posterior to ovary; usually not joining along posterior margin of segment, but in 1 in 3 of fully mature segments testes joining over posterior dorsal surface of vitelline gland. Eight to 11 testes in poral group, 12 to 16 in aporal group. Testes 0.04 to 0.05 in diameter, Genital atrium small, inconspicuous, opening on lateral margin in anterior third of segment; alternating irregularly; tendency of 5.3 to open on left.

Dorsal excretory vessels 2 to 3 microns in diameter, usually within or immediately above ventral excretory vessels measuring 7 to 8 microns in diameter.

Cirrus-sac 0.18 to 0.20 long by 0.03 to 0.04 wide, passing between lateral excretory vessels and containing a coiled internal vesicular seminalis. Vas deferens convoluted in anterior part of segment.

Vagina opening into genital atrium posterior to cirrus-sac, narrowing after proximal portion, passing between lateral excretory vessels, and expanding to form receptaculum seminis in mid-line. Ovary lobed, median. Vitellaria postovarian, median, compact, 0.05 to 0.06 in diameter. Uterus ventral; arising anteriorly as a small sac, quickly branching, and filling segment; persistent; always remaining within lateral excretory vessels. A U- or Y-shaped central fold often visible in early gravid segments. Eggs 45 to 52 microns in diameter. Embryonic hooks 16 to 18 microns long.

Host: Crested Barbet, *Trachyphonus vaillantii* (Ranzani)

Location: Intestine.

Locality: Fort Tuli Southern Rhodesia.

Co-type to be deposited in the British Museum (Natural History).

DISCUSSION

Because of its persistent uterus the present material falls within the sub-family Dilepidinae Fuhrmann, 1907.

Of the genera in this sub-family only one, *Arctotaenia* Baer, 1956, possesses an unarmed scolex and no rostellum. Yamaguti (1959) considerably expanded Baer's (1956) generic diagnosis of *Arctotaenia* by incorporating details of the type species. The present material differs from Baer's description in that the genital pores alternate irregularly, the female genitalia are median and slightly pre-equatorial in position, the testes tend to be in two lateral groups,

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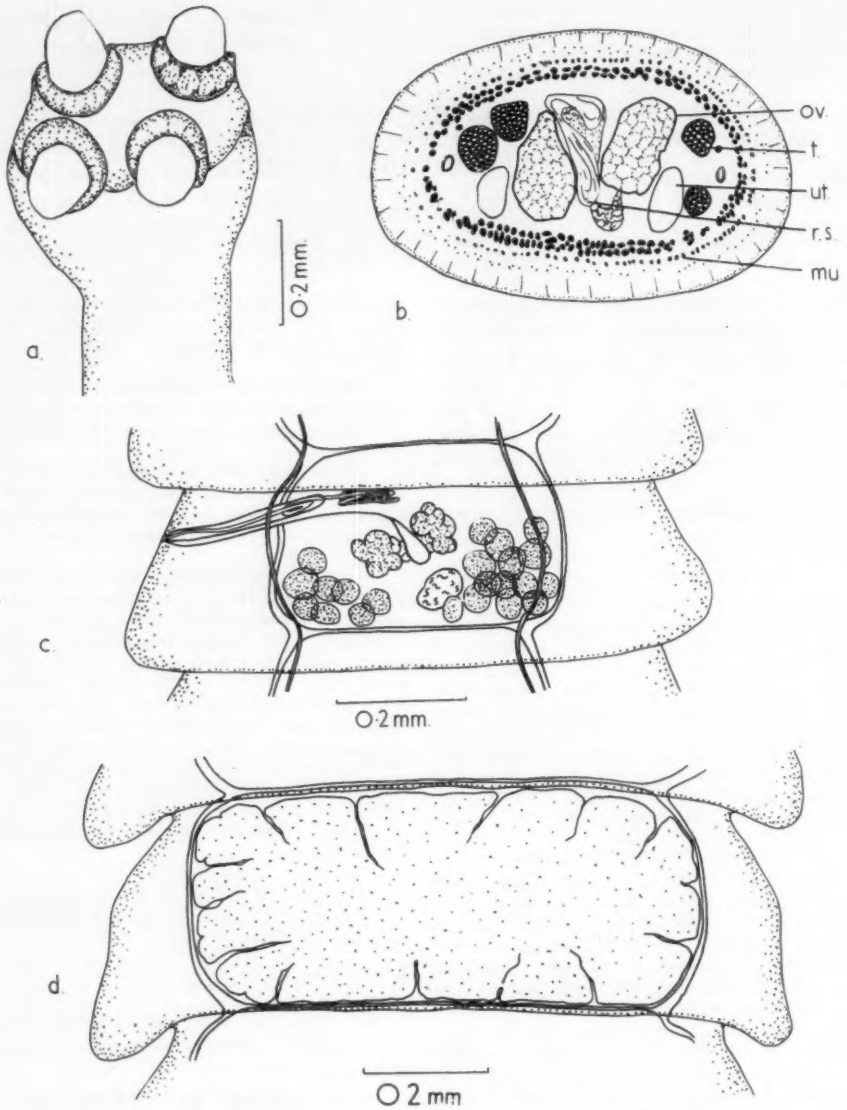

FIGURE 1. *Ethiopotaenia trachyphonoides* gen. n., sp. n. a, scolex; b, t.s. mature segment; c, mature segment; d, gravid segment.

LEGEND TO FIGURE: ov, ovary; t, testis; ut, uterus; r.s, receptaculum seminis; mu, outer longitudinal muscle layer.

and the uterus is always confined between the lateral excretory vessels.

Unciunia Skrjabin, 1914, has a rudimentary unarmed rostellum, and in addition may be distinguished by possessing a very deep genital atrium, a collar of spines at the base of the cirrus plus a chitinous hook, testes arranged behind the ovary, and the uterus divided into compartments.

ACKNOWLEDGMENT

The Author is indebted to Mr. Stephen Prudhoe of the British Museum (Natural History) for advice on the preparation of this paper.

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RESEARCH NOTE

TRITRICHOMONAS BATRACHORUM IN A HOG-NOSED SNAKE.

In connection with a study being carried out under the direction of Dr. Deam H. Ferris of the College of Veterinary Medicine, University of Illinois, the organs of a large number of wild vertebrates have been cultured for *Leptospira*. In a culture inoculated in September 1960 with the liver and kidney of an eastern hog-nosed snake, *Heterodon platyrhinos* Latrielle, a trichomonad was detected by Mrs. Rachel Marlowe. The snake had been trapped in southern Illinois in the vicinity of the University of Illinois Dixon Springs Experiment Station, Robbs, Illinois. The trichomonad multiplied in both Stuart's and Fletcher's *Leptospira* media (Difco) containing 6 to 10 percent inactivated rabbit serum. At the time the cultures were made extreme care was exercised to prevent any contamination with the intestinal contents, and it is therefore assumed that the trichomonad had been present in either the liver or the kidney or both. Subcultures were made to several different culture media, but it was soon found that the organism grew best in Lash's medium (Difco), and subsequent transfers were made only in this medium, approximately every 3 weeks. The trichomonad grew best at room temperature (approximately 25 C) and would not survive at 37 C.

This parasite was studied by phase-contrast microscopy and also with a Leitz Ortholux microscope equipped with apochromatic objectives. It was stained with Honigberg's (1951, University of Calif. Publ. Zool. 55: 337–394) modification of the Bodian silver stain following fixation with Hollande's fluid, with Heidenhain's hematoxylin following fixation with Schaudinn's fluid, and with Giemsa's stain following fixation with methanol. It was identified as *Tritrichomonas batrachorum* (Perty, 1852) Honigberg, 1953.

The protozoa are broadly ovoid (fig. 1) and have three anterior flagella of unequal length. A costa can be seen in nearly all silver stained specimens. A row of paracostal granules is clearly visible under phase-contrast. The axostyle protrudes posteriorly about two-thirds of the body length, tapering gradually to a point. No granules are visible in the axostyle. The undulating membrane has several prominent undulations and stains clearly with both silver

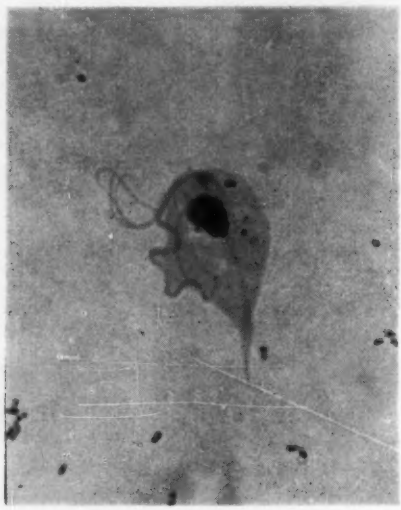

Figure 1. Photomicrograph of *Tritrichomonas batrachorum* from *Heterodon platyrhinos*. Culture form in Lash's medium. Methanol, Giemsa. × 2000. Note costa. The free posterior flagellum is not visible.

and Giemsa stains. A free posterior flagellum continues beyond the undulating membrane; it is usually about three-fifths as long as the body. The parabasal body, visible in some of the hematoxylin stained individuals, is shaped like an inverted "V." Thirty trichomonads were measured with the phase-contrast microscope after fixation in 2 percent osmic acid vapor. Their dimensions in microns (means followed by ranges in parentheses) were: Length, 10.8 (8.3 to 13.0); width, 8.0 (5.7 to 10.9); anterior flagella, respectively, 9.8 (6.8 to 13.5), 14.0 (10.9 to 16.1), and 17.6 (14.6 to 19.2); protruding portion of axostyle, 7.4 (5.2 to 10.4); and length of trailing flagellum, 6.3 (4.7 to 8.8). These measurements are similar to those given for *Tritrichomonas batrachorum* by Honigberg (1953, J. Parasit. 39: 191–208) and for one of the strains studied by Samuels (1957, J. Prot. 4: 110–118).

Honigberg (1953, J. Parasit. 39: 191–208) and Morgan (1948, Bovine Trichomoniasis. Burgess Publishing Co., Minneapolis 15, Minn.) listed several snakes other than *Heterodon* spp. as hosts of *Tritrichomonas batrachorum*, and Trussell (1947, *Trichomonas vaginalis* and Trichomoniasis, Charles C Thomas Publishing Company, Springfield, Ill.), in his trichomonad parasite-host list, stated that *Trichomonas* had been reported from *Heterodon simus*, the southern hog-nosed snake. Lee (1960, J. Prot. 7: 393–401) reported *Hypotrichomonas acosta* from *Heterodon platyrhinos*, but *Hypotrichomonas* differs from *Tritrichomonas* in that it lacks a costa.

This is apparently the first record of *T. batrachorum* in *Heterodon platyrhinos*. It is also the first report of which we are aware of this trichomonad in organs other than the intestine. It is noteworthy that this flagellate grew successfully in culture media prepared specifically for *Leptospira*.

This investigation was supported by research grants (E-790 and in part, E-3077) from the National Institutes of Health, Public Health Service—FERRON L. ANDERSEN AND NORMAN D. LEVINE, *College of Veterinary Medicine and Agricultural Experiment Station, University of Illinois, Urbana.*

THE LABORATORY PROPAGATION OF *SPIROMETRA MANSONOIDES* AS AN EXPERIMENTAL TOOL. V. BEHAVIOR OF THE SPARGANUM IN AND OUT OF THE MOUSE HOST, AND FORMATION OF IMMUNE PRECIPITATES

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The plerocercoid or sparganum of *Spirometra mansonoides* has been maintained and studied in this laboratory for a number of years, in culture media and in the white mouse and other experimental animals (Mueller, 1959). In mice infected early in life, at a weight of 10 to 15 g with procercoids per os, as many as 150 larvae may develop in a single mouse, reaching a length at the end of a year of 200 to 400 mm per worm, and forming as much as 17 percent of the weight of the animal (Mueller, 1958). When such mice are posted, the subdermal spaces and intermuscular fascial planes are cluttered with great masses of spargana. Usually these worms have only very light encapsulation, insufficient to prevent migration. At times it appears that a wall of host tissue is entirely lacking. Nevertheless the worms lie in small piles, often discrete, but also frequently touching (figs. 1, 2). However, they never become knotted or tangled in the mouse. It is therefore easy to remove them individually with a fine forceps to a dish of saline for counting, measuring, etc.

Outside the body of the mouse, whether in saline or culture medium, at room temperature or 37 C, the worms show constant slow peristalsis, with waves of contraction and extension passing along the body from anterior to posterior. If a number of worms are left in the same dish, they quickly become knotted and tangled together, so that it is necessary to disperse them in different dishes, and make observations soon after recovery from the host. Otherwise, they become hopelessly snarled. When 50 or so large spargana (200 mm or more in length) are placed together in a saucer, in either saline or CS-199 (basic medium without chick embryo extract), and left for some hours, they become so tightly knotted that the snarl rises above the level of the fluid in the dish. The tangled bodies and tails form this knot, while the heads radiate in all directions, continuing their peristaltic probing movements (figs. 4, 5).

When either procercoids or spargana are fed to mice, or when sparganum heads are injected subcutaneously or intraperitoneally, they migrate through the body, sometimes considerable distances, since they may occur almost anywhere. But from the appearance of the neat little piles which individual worms present in the posted mouse, it would appear that once having gained a suitable position, migratory activity ceases, and the worm grows, doubling back and forth as its length increases. Thus it appears that the behavior of the worm during the first month or so in the mouse differs markedly from its subsequent behavior when it has settled down in a particular location.

The first month, which is the period of antibody formation in the mouse, is also the period of migration and dispersion. But apparently some factor in the mouse serves to quiet the worm and stop migration after this initial period. It seemed reasonable to suppose that antibodies, or some other factor in the serum of infected mice, might have this effect, particularly since worms reared in culture over a period of months often grow at a rate comparable to that in the mouse, but continue their crawling movements indefinitely.

MATERIALS AND METHODS

On this hypothesis, blood serum was collected from a series of mice which had been infected for a period of several months to 1 year (E-38b, C-52, C-81). The sera were not heat-inactivated, and no preservative was added, since merthiolate is lethal to the worms even in dilutions of 1 to 10,000. Blood serum from normal uninfected mice, culture medium (CS-199-CEE), and saline were used as controls.

One ml of serum or culture medium was placed in small tubes, and a sparganum (from 10 to 15 mm long, reared in culture, and washed in saline) placed in each of the tubes. Usually, four or five tubes were set up at a time, using the immune mouse sera, normal mouse serum, and

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culture medium. These tubes were stoppered with corks and incubated at 37 C, and examined for motility at 6, 12, 24, and 48 hours.

RESULTS

In no case was activity of the worm arrested, and the appearance of the worms in immune serum was essentially similar to that of worms in non-immune serum or culture medium. Before discarding the worms, they were examined under the microscope between a slide and cover slip. For the first time, it was then apparent that in immune sera a precipitate had formed along the length of the worm. This was fully evident as early as 6 hours incubation. This precipitate was lighter and more flocculent in the body region, but tenacious and often serrated in profile in the head region. Occasionally, this serrated appearance extended the greater length of the worm. The posterior tip of the sparganum was also enclosed in a thick cap of precipitate. On the head, the precipitate was thickest in the region of the bothria, or just posterior, and thinnest at the tip of the scolex. In 24- and 48-hour worms, much precipitate had fallen away and lay in the bottom of the tube.

In both normal serum and culture medium, the worms were free of precipitate along the greater part of their length, but in the region of the head a precipitate formed which was not markedly dissimilar to that found in immune sera (figs. 6. 8).

DISCUSSION

Precipitates of this latter kind were mentioned and figured in my earlier paper (Mueller, 1959, fig. 9). This type of precipitate, which has nothing to do with immunity, forms a collar around the neck and scolex of worms growing in culture. It adheres tenaciously to the worm, but after reaching a size several diameters larger than the worm itself, it drops off. These collars of precipitate have to be removed periodically from cultures, and when examined can be seen to form a perfect cast or mold of the scolex, with its bothria (figs. 9, 10, 11). Such precipitate collars do not form on worms kept in saline. They are thus produced not by the worm alone, but by interaction of the worm and protein in the medium.

Glands have been described in the scolex and bothria of various adult pseudophyllidean worms, notably *Adenocephalus* and *Glandicephalus* (*see* Wardle and McLeod, 1952), and are probably present in all. Obviously, at least some of these glands produce a coagulating substance. It is not clear what purpose such glands serve in the plerocercoid, living as it does in the tissue spaces. But in the adult in the intestinal lumen they might function as an additional holdfast device, cementing the scolex in place and forming an additional obstacle to the removal of this vital part, so that tension applied to the worm tends to sever the neck rather than to remove the scolex.

Whether this precipitate along the length of the worm is a true antigen-antibody reaction in the classical sense is not certain. But this seems probable from the fact that it occurs only in the serum of chronic infected mice. A caseous substance is often found around worms removed from the mouse host, and is no doubt in part this same precipitate.

The formation of precipitates at body orifices, when parasitic nematodes or their larvae are exposed to homologous antisera, has been known and investigated since Sarles (1938) first described the phenomenon for *Nippostrongylus muris*. Such precipitates occur also in trematodes similarly treated, and have been particularly studied in the case of schistosomes, where they account for the familiar CHR and circumoval reactions, and also cercaricidal and agglutinative effects. Kagan (1958) has given a general review of this subject. Immune reactions have received less attention in cestodes, but Heyneman and Welsh (1959) have reported precipitates on young adults of *Hymenolepis nana* exposed to homologous rabbit antiserum. The precipitates figured are very similar to those reported in this paper for *Spirometra*. Heyneman and Welsh noted that young adult worms in immune serum were more active, even after 48 hours, than worms placed in normal rabbit serum. This agrees in general with my observation that immune serum does not quiet the sparganum.

The occurrence of heavy precipitate on the tail in my experiments is of interest because the excretory ducts open at this location. Thus we have a heavy precipitate occurring at or in the region of a natural orifice of the worm.

Mueller (1960) has described the effect on sparganum larvae when incubated with the serum of fish naturally resistant to infection

with *Spirometra*. In some fishes a factor is present in the serum which coagulates and strips the cuticula from the worm. In others, this factor is lacking in serum, but when worms are implanted into the fish they are rapidly attacked and destroyed by phagocytosis. This type of natural immunity differs from what is observed in the mouse, in that in the latter host the cuticula is not eroded, and the worm is not killed.

The original question as to why spargana remain quiescent in the mouse host after the initial stage of the infection remains unanswered, but apparently more is involved than the presence of some factor in the serum of chronic infected mice. Possibly physical rather than chemical factors are involved.

This investigation was largely supported by continuing grants from the National Institutes of Health, U.S.P.H.S., No. E-1876.

SUMMARY

Sparganа in culture medium continue to execute peristaltic crawling movements indefinitely. In the mouse host, on the other hand, after an initial period of migration, corresponding to the period of "immunization," the worms settle down, so that even large numbers in a single mouse never become knotted together. On the hypothesis that this change in behavior might be a reaction to antibodies or some other substance in the blood of "immune" mice, spargana were incubated with serum from chronic infected mice, using normal mouse serum and culture medium for controls.

No difference in motility was noted at any time up to 48 hours incubation. But precipitate formed along the body of worms in immune serum, and was well developed after 6 hours. This is presumably an immune precipitate since it does not form in normal mouse serum or in culture medium. This precipitate differs from one which forms on the head of worms in culture medium or normal serum, and which in culture is cast off periodically in the form of a collar. This precipitate is obviously the work of coagulating glands in the scolex. Function of these in the larva is problematical, but in the adult they probably serve as an additional holdfast device, cementing the scolex into the intestinal mucosa of the host, and thus augmenting the adhesive action of the bothria.

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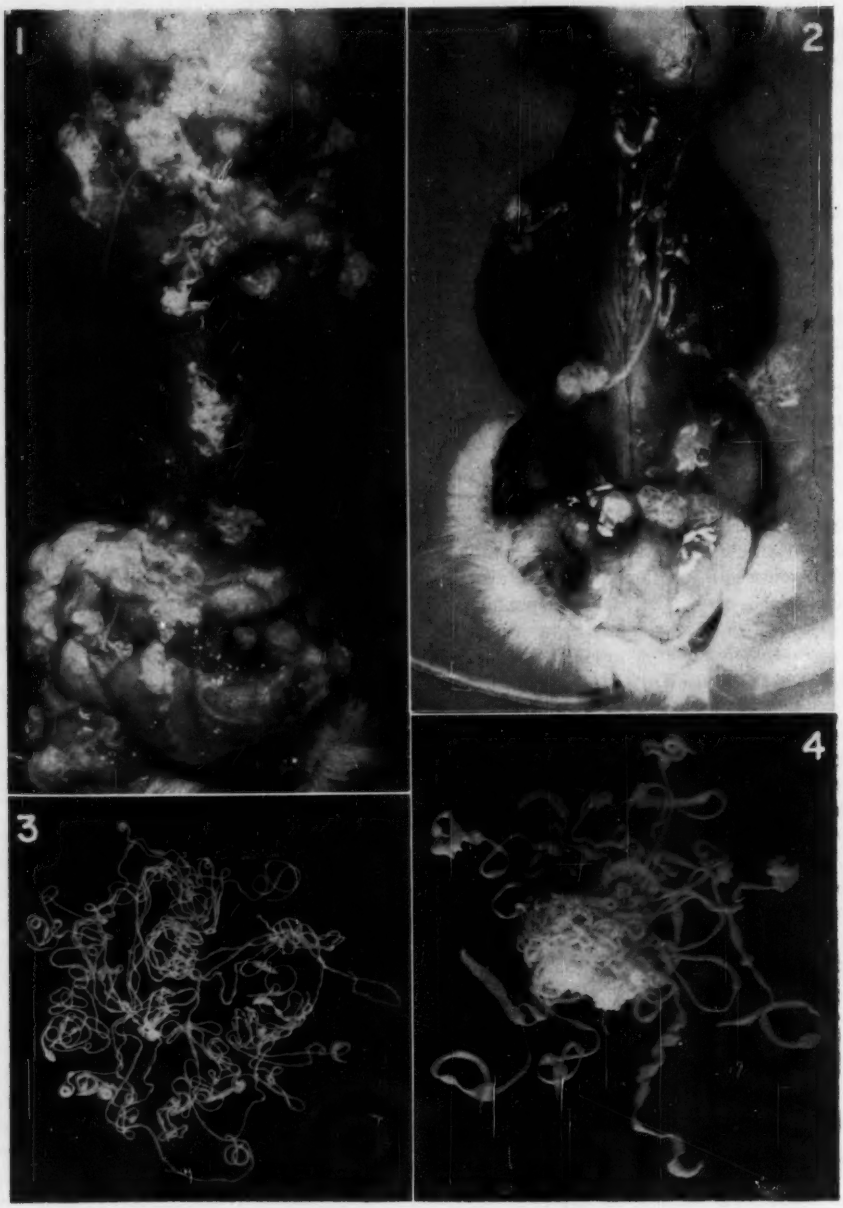
PLATE I



FIGURES 1, 2. Sparganum-infected mice, skin snipped at waist and drawn toward both ends; showing tendency of worms to lie in discrete small piles.

FIGURE 3. Sixteen worms removed from mouse shown in figure 2 (C-75, 12–28–59), photographed immediately on removal to dish of saline.

FIGURE 4. Worms shown in figure 3, twelve hours later, now knotted into a ball.

PLATE II

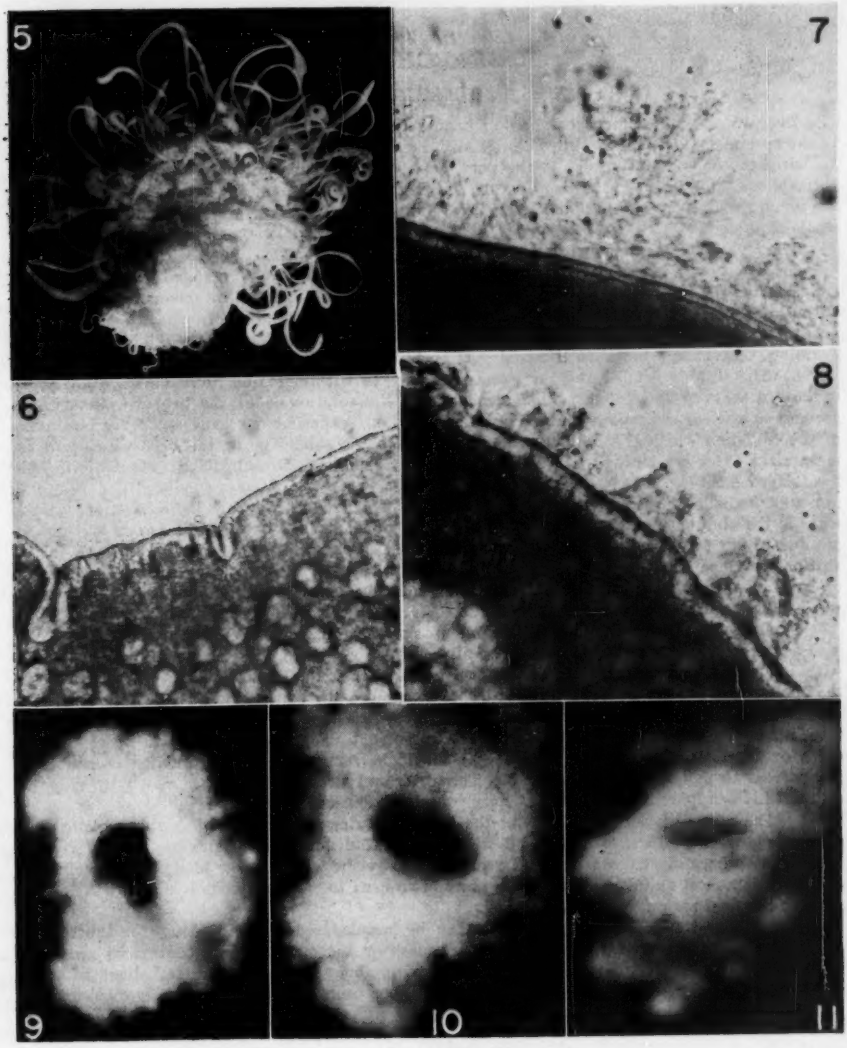


PLATE II

FIGURE 5. 153 worms removed from mouse shown in figure 1 (C-37, 3–23–58), weighing 8.3 g and constituting 17.87 percent of the total weight of the animal. After standing and knotting.

FIGURE 6. Cuticula of sparganum after incubation in normal mouse serum for 6 hours or more. No precipitate.

FIGURE 7. Cuticula of sparganum after 6 hours incubation in serum of infected mouse, showing flocculent amorphous precipitate clinging to worm.

FIGURE 8. Anterior region, showing serrated profile of thick precipitate clinging to cuticula.

FIGURES 9, 10, 11. Rings of precipitate which form on head of worms in culture, showing mold of the scolex and bothria.

RESEARCH NOTE

TECHNIQUES FOR HANDLING TREMATODES.

The preparation of large numbers of trematodes for study presents a number of problems. If the specimens are of moderate to large size, i.e., more than 2 mm, the procedure of staining, dehydration, and mounting is, at the very least, time consuming. With the small or minute forms (less than 2 mm), the problems of staining, differentiation, dehydration, and mounting are much greater. The techniques described below shorten considerably the time used to make whole mounts.

The technique for large worms is as follows:

1) The worms are removed from 70 percent ethyl alcohol and arranged on a microscope slide just as they are to be arranged in the permanent mount. If the specimens are curled and with a heavy musculature, it is best to flatten them with pressure between two slides in 85 percent alcohol until they retain the flattened shape. The excess alcohol should be removed by draining. Care should be taken to avoid getting lint among the specimens.

2) The slide is flooded and the worms are covered with collodion diluted with equal parts of both ether and 100 percent ethyl alcohol until the solution is of the proper viscosity. On account of the rapid evaporation of the ether, one must experiment to find the correct dilution of the collodion which has been in storage. The collodion should be of the type which will remain clear after hardening so differentiation can be controlled easily. The collodion which is not labeled flexible seems to be satisfactory.

3) The collodion is hardened in 85 percent alcohol until both the collodion and the worms slip off the slide easily. Hardening can be carried out conveniently in a beaker if the film is allowed to dry sufficiently so the specimens will not slip or move when the slde is placed in the vertical position. If the trematodes are of the flaccid type or if they were flattened at the time of fixation, the slide-side of the collodion film should be inspected for secure placement of the worms. Sometimes a thin coat of collodion is required on this side.

4) As many as 100 of these collodion mounts with worms in them can be placed in a Petri dish for processing. Staining is best accomplished in dilute stain (Harris' haematoxylin diluted with 10 percent ammonium alum for several hours duration). If progressive staining is used, the specimens are passed through acid-alcohol to remove the small amount of stain which the collodion picks up. During dehydration the higher alcohols should be changed at least twice. One-half hour is sufficient at each change. The 95 percent alcohol is replaced with a 1:1 mixture of cedar wood oil and 95 percent alcohol. It should be remembered that collodion is soluble in 100 percent alcohol, methyl salicylate, etc. The water and alcohol are removed slowly by evaporation until it is apparent that the worms are completely cleared.

5) The edges of the collodion and the label are trimmed easily with a razor blade. The excess cedar wood oil can be rinsed off with xylene. If a number of slides are desired from a single sheet of worms, the worms can be cut out one-by-one with a razor blade. If the collodion film is made almost as thick as the worm, there is no need to use glass rods to support the coverglass in the final mount.

Trematodes smaller than about 2 mm can be handled readily by making for them small receptacles which are covered with a fine wire mesh on the one end. The containers are made from polyethylene tubing with an inside diameter of about 8 mm and are cut about 10 to 12 mm long. Wire mesh with about 100 wires per inch has been found to be satisfactory (stainless steel, purchased from Newark Wire Cloth Co., 351 Verona Ave., Newark, N. J.). Circles of the wire cloth are cut to the proper diameter and placed on a hotplate. The wire mesh is then sealed to the polyethylene by means of heat. These containers can be used in a Petri dish where the fluids can be changed easily. Dehydration can include 100 percent alcohol, and xylene should be used for clearing. The worms are removed from the receptacle by means of a medicine dropper. If the dropper is held in a vertical position and rotated slowly, the worms will settle to the bottom where they can be placed on the slide in a single drop. The excess xylene is removed; mounting is carried out as usual.—WILLIAM H. COIL, *Department of Zoology, University of Nebraska, Lincoln.*

DESCRIPTION AND LIFE CYCLE OF *RAILLIETINA GEORGIENSIS* N. SP., A TAPEWORM FROM WILD AND DOMESTIC TURKEYS*

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In November 1958 a flock of 1500 domestic turkeys on a farm at Winterville, Georgia, was found to be heavily infected with tapeworms. Specimens are being described as a new species, *Raillietina* (*Raillietina*) *georgiensis*. In a preliminary report these worms were referred to *R. williamsi* (Reid and Nugara, 1959). Several breeds of domestic turkeys and one wild turkey on the farm were found to be infected. Six wild turkeys had been introduced to the flock some months previously from a hatchery operated at Fitzgerald, Georgia, by the State Game and Fish Commission. Since wild turkeys reared at the hatchery were subsequently proved to be infected with this same species of tapeworm it is considered possible that the parasite was introduced to the farm with the six wild turkeys.

Examination of 500 intestines between November 1958 and January 1959 revealed 90 percent of the domestic turkeys to be infected with from 1 to 175 tapeworms each. Immature worms were found attached to the intestine from the duodenum to the ileum with largest numbers in the middle third of the intestine. Mature worms occurred more frequently in the posterior third of the alimentary tract. With the exception of the wild turkeys, by the middle of January 1959 all the birds on the range had been sold. Young poults were put on the same range the following July and by mid-August, 4 out of 20 birds had become infected with a total of 20 worms being recovered. The incidence of this infection increased until nearly 100 percent of the young turkeys were parasitized.

Cysticercoids of *R. georgiensis* have been recovered from a small ant belonging to the species *Pheidole vinelandica* Forel** Subsequent experiments showing development of mature worms from the cysticercoids demonstrated the life cycle.

Raillietina (*Raillietina*) *georgiensis* N. Sp.

Description of the adult tapeworm: Fully developed strobila measuring 150 to 380 mm in length with maximum breadth of 3.5 mm; rostellar hooks numbering 220 to 268 (av. 231) measuring 17 to 23 microns in length by 12 to 16 microns in width, arranged in two rows. Suckers approximately round, 110 to 179 microns long by 80 to 151 wide; sucker hooks distinctive (fig. 5), arranged in 8 to 10 circles; hook length 8 to 13 microns with longer ones in outer circles. Neck 1.3 to 3.2 mm long. Immature and mature segments greater in width than in length. Gravid proglottids longer than broad when shed. Genital pores unilateral, rarely irregularly alternate; situated in middle third of segment (fig. 9). Testes 23 to 29 in number (fig. 14) round or irregular with an average diameter of 65 microns; distributed in two groups 7 to 9 poral and 16 to 20 aporal; lying between excretory canals. Sperm duct arising at center of segment, extending laterally with many convolutions, to base of cirrus sac. Cirrus sac small, measuring 96 to 143 microns long and 55 to 96 microns wide extending one-half distance to longitudinal excretory canal; cirrus unarmed with three coils surrounded by cirrus sac when in retracted condition. Ovary central in mature proglottids, occuping entire anterior-posterior length of proglottid; num-

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** Identification of species was made by Dr. Marion R. Smith of the U.S. National Museum, Washington, D.C.

ber of lobes 3 to 10 varying with maturity. Vitelline gland smooth, 200 to 248 microns long by 82 to 110 microns wide located posterior and medial to ovary. Unconvoluted vagina parallel to and closely posterior to sperm duct, entering genital atrium posterior to male system. Vagina in some specimens showing definite enlargement near atrium similar in size to cirrus sac. Vagina passing dorsal to ventral excretory canal. Gravid proglottids containing 80 to 130 egg capsules, each with 8 to 10 eggs; capsules irregular in shape and diffused. In older proglottids one to two rows of capsules found lateral to excretory canal (fig. 15). Diameter of eggs 27 to 48 microns; diameter of oncosphere 17 to 20 microns; oncosphere hook 5 to 7 microns long. Type specimens No. 55868 in the U. S. N. M. Helm Col., Parasitological Laboratory, USDA, Beltsville, Maryland. *Type host: Meleagris gallopavo silvestris,* also found in *Meleagris gallopavo domesticus. Type locality:* Winterville, Georgia.

Description of the cysticercoid. Mature cysticercoid (fig. 16) oval, milky white in color, 220 to 351 microns long and 172 to 289 microns broad. External covering of three layers surrounding cysticercoid cavity. Outer layer transparent, 3 to 7 microns in thickness. Suckers 55 to 96 microns long, 41 to 69 microns wide; hook zone 13 to 20 microns; 8 to 10 rows of acetabular hooks; each hook 8 to 13 microns long, 3 to 4 microns wide. Rostellum 55 to 96 microns in diameter with 162 to 256 rostellar hooks arranged in two rows; hook length 17 to 23 microns long, 12 to 16 microns wide.

Host of cysticercoid: Pheidole vinelandica Forel.

Taxonomic Relationships

Because of the similarities in appearance, hosts, and geographical distribution *Raillietina* (*Raillietina*) *georgiensis* appears to be most closely related to *R. williamsi* Fuhrmann, 1932. *R. williamsi* has been reported from the wild turkey from Pennsylvania and Maryland (Gardiner and Wehr, 1949) and has been found in the present studies from the Shelby National Forest area near Memphis, Tennessee. *R. georgiensis* differs from *R. williamsi* in having more and smaller rostellar hooks. With the former species the average number is 231 hooks measuring 17 to 23 microns as compared to 168 (range 142 to 202) hooks measuring 28 to 34 microns in our restudy of *R. williamsi.** Williams (1931) reported 155 hooks measuring 33 to 38 microns with this latter species. The genital pore is located in the middle third of the proglottid in *R. georgiensis* while it is in the anterior third in *R. williamsi* (figs. 9 and 10). Moreover, the strobila of *R. georgiensis* is somewhat narrower with a maximum width of 3.0 to 3.5 mm compared with 3.5 to 4.2 for *R. williamsi.*

Similarly *R. georgiensis* may be differentiated from *R. tetragona* and *R. echinobothrida* by differences in rostellar hook size and number, and in location of the genital pore. In the latter two species the number averages 100 and 200, respectively. The hooks measure 6 to 9 and 10 to 13 microns in length (figs. 1, 3, 4). The genital pore is in the anterior third in *R. tetragona* and the posterior third in *R. echinobothrida.* Horsfall (1938) and Sawada (1952) have found that the hook length in the cysticercoid is within the same range as that of the adult in both the species *R. tetragona* and *R. echinobothrida.* When comparison is made between larval and adult hooks of *R. georgiensis* the size is found to be the same. These findings indicate the reliability of hook size as a species characteristic. A restudy of the location of the genital pore in 4 species using the method of Sawada (1954) has been completed with species differences recorded above (fig. 9, 10, 11, 12). These results confirm Sawada's conclusions but indicate a more anterior position for *R. tetragona* than that reported earlier by Ransom (1905), Southwell (1930), and Horsfall (1938). It is assumed that a typographical error is responsible for the reversal from the usually accepted positions as recorded in table I of the latter investigator.

Eleven other species of cestodes reported from Galliformes including *R. multicapsulata* (Baczyńska, 1914), *R. cohni* (Baczyńska, 1914), *R. shantungensis* Winfield and Chang, 1936, *R. volzi* (Fuhrmann, 1905), *R. penetrans* (Baczyńska, 1914), *R. pintneri* (Klaptocz, 1906), *R. ceylonica* (Baczyńska, 1914), *R. friedbergeri* (Linstow, 1877), *R. peradenica* Sawada, 1957, *R. grobbeni* Böhm, 1925, and *R. graeca* Davies and Evans, 1938, may be differentiated from

* New observations have been made on *R. williamsi* from preserved slides loaned by Dr. E. E. Wehr of the USDA at Beltsville and from new specimens from Tennessee. The original type specimens at the Philadelphia Zoological Garden have been lost.

R. georgiensis by smaller rostellar hooks and other characteristics such as number of testes, eggs per capsule, size of cirrus pouch, or general body size.

Some recent descriptions of species from this genus have limited differential characteristics to species found within a single host. However, *R. tetragona* which is related to *R. georgiensis* has also been reported from several species including the three orders Galliformes, Columbiformes, and Anseriformes. In the present work comparison has been made between *R. georgiensis* and 158 other species of the subgenus *Raillietina*. It differs in size and number of rostellar hooks from all other species recorded from birds except for 6 from Columbiformes and two species from Psittaciformes. The three species *R. columbiella* Ortlepp, 1938, *R. perplexa Johri*, 1933, and *R. insignis* (Steudner, 1877) from Columbiformes differ from *R. georgiensis* in general size, number of oncospheres per capsule and descriptions of the cirrus sac. *R. nagpurensis* Moghe, 1925, has 19 to 22 testes; *R. frayi* Joyeux and Houdemer, 1927, has 18; and *R. joyeuxi* (Lang, 1929) has 45 to 60 testes compared with 23 to 29 in *R. georgiensis*. *R. allomyodes* (Kotlán, 1921) of the Psittaciformes differs in having an armed cirrus and fewer testes, (12 to 16), while *R. psittacea* (Fuhrmann, 1911) has fewer hooks (160) and the egg capsules do not extend beyond the longitudinal excretory vessels.

Infection Experiments from Naturally Infected Ants

In the preliminary search for intermediate hosts ground beetles, earthworms, crickets, and ants were collected from open range and from nearby wooded areas. These were examined for cysticercoids. Only from the small ant, *Pheidole vinelandica* Forel, were cysticercoids recovered. The number ranged from 1 to 8 in worker ants and 1 to 23 in soldier ants. Examination of 2000 ants from the farm during October and November, 1958, revealed an infection rate of 3.5 percent. During the cold winter months from December to March attempts to collect ants were unsuccessful. In the spring while turkeys were not on the range, the percentage of infection in ants was gradually reduced: 2.7 percent of 222 ants in April, 0.7 percent of 1486 ants in May and 0.1 percent of 846 ants in June. During this period there was a gradual increase in the number of the cysticercoids which showed some stage of degeneration resembling the phenomenon reported by Sawada (1953) for another species which has a cysticercoid stage in ants. This investigator indicated that the longevity of the cysticercoid of *R.* (*Paroniella*) *kashiwarensis* was relatively short.

The cysticercoids were freely visible in the abdominal cavity of the ant without dissection. The technique for intact in vivo identification was to flatten the abdomen under a coverglass by pressure from a dissecting needle while examining the ant under low power of the microscope. Ants of the same species from the same or nearby farms were found infected with cysticercoids identified by the distinctive hook pattern as larvae of *R. tetragona* or *R. echinobothrida*.

The first cysticercoids of *R. georgiensis* recovered were fed to domestic turkeys which had been reared on wire floors in laboratory isolation. Two 6-month-old turkeys which had been given 25 cysticercoids per os by gelatin capsule were positive with these seven adult tapeworms respectively, after 20 days. Likewise two out of three day-old poults were later positive with three and five worms respectively, after receiving 15 cysticercoids each. Although attempts to recover the first terminal gravid proglottid failed, the minimal maturing time is known to be less than 3 weeks. Normal cysticercoids recovered during the spring months were fed to chickens in order to determine whether or not the worm could be established in these hosts. One to seven cysticercoids were fed to each of seven chickens in April and five other chickens in May. All were negative for tapeworms upon necropsy. From one to four cysticercoids recovered in May or June were fed to each of seven turkey poults. One of the birds became infected with an adult worm thus showing that some of the late spring cysticercoids were still viable. The tapeworm *Staphylepsis carioca* (= *Hymenolepis carioca*) and *R. tetragona* were recovered from 100 chickens off the same range, but all were negative for *R. georgiensis*. Although these studies do not prove that this species cannot infect chickens, they certainly suggest that the turkey is the preferred host.

SUMMARY

1. A new species of tapeworm, *Raillietina (Raillietina) georgiensis*, is described from domestic and wild turkeys near Winterville, Georgia. The domestic flock of turkeys appears to have been infected by introduction of wild turkeys from the Georgia State Farm and Fish Commission hatchery near Fitzgerald, Georgia. Over 90 percent of 1500 in a flock of domestic turkeys were infected with 1 to 175 worms each.

2. The small ant, *Pheidole vinelandica* Forel, harbored 1 to 23 cysticercoids in the abdominal cavity. The adult tapeworm was recovered after feeding cysticercoids to turkeys reared in laboratory isolation. The tapeworm does not appear to infect chickens, since infection trials were unsuccessful and 100 chickens taken from the infected range area were negative for *R. georgiensis*.

3. *Raillietina* (*Raillietina*) *williamsi* is reported from a game-management area near Memphis, Tennessee.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the following: Dr. Everett E. Wehr of the Animal Disease and Parasite Research Division, United States Department of Agriculture, Beltsville loaned specimens of *Raillietina williamsi;* Dr. Elon Byrd, Zoology Department, University of Georgia and Dr. Marion R. Smith, U. S. National Museum, Washington, D. C., identified the ant specimens; George C. Moore of the Georgia State Game and Fish Commission cooperated in studies of wild turkeys at Fitzgerald, Georgia; Robert D. Smith and James Lewis, Tennessee Game and Fish Commission, assisted in a study of wild turkeys infected with *R. williamsi;* Dr. Burton G. Maxfield assisted in life history studies involving chickens and turkeys.

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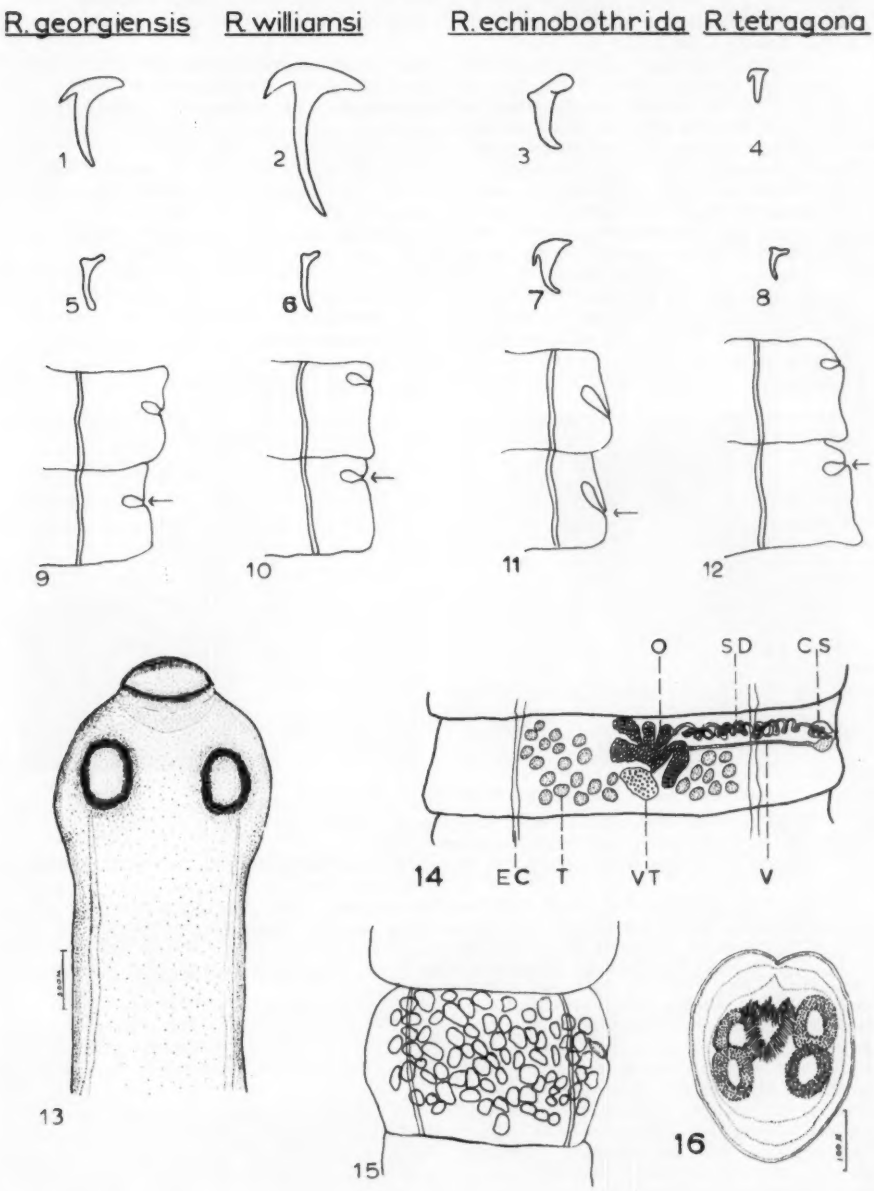
EXPLANATION OF PLATE I

Figure 1. Rostellar hook of *R. georgiensis*, n. sp. (The hooks from adults tapeworms in figures 1 to 8 are drawn to the same scale.)
Figure 2. Rostellar hook of *R. williamsi*, Fuhrmann, 1932.
Figure 3. Rostellar hook of *R. echinobothrida* (Mégnin, 1880).
Figure 4. Rostellar hook of *R. tetragona* (Molin, 1858).
Figure 5. Acetabular hook of *R. georgiensis*.
Figure 6. Acetabular hook of *R. williamsi*.
Figure 7. Acetabular hook of *R. echinobothrida*.
Figure 8. Acetabular hook of *R. tetragona*.
Figure 9. Location of genital pore *R. georgiensis*.
Figure 10 Location of genital pore *R. williamsi*.
Figure 11. Location of genital pore *R. echinobothrida*.
Figure 12. Location of genital pore *R. tetragona*.
Figure 13. Scolex of *R. georgiensis* with rostellum extended.
Figure 14. Mature proglottid of *R. georgiensis*. O, ovary; S.D., sperm duct; C.S., cirrus sac; E.C., excretory canal; T., testis; V.T., vitelline gland; V., vagina.
Figure 15. Gravid proglottid of *R. georgiensis* showing position of egg capsules in relation to excretory canals.
Figure 16. Cysticercoid of *R. georgiensis* which has been dissected from the abdominal cavity of an ant.

PLATE I


R. georgiensis
R. williamsi
R. echinobothrida
R. tetragona
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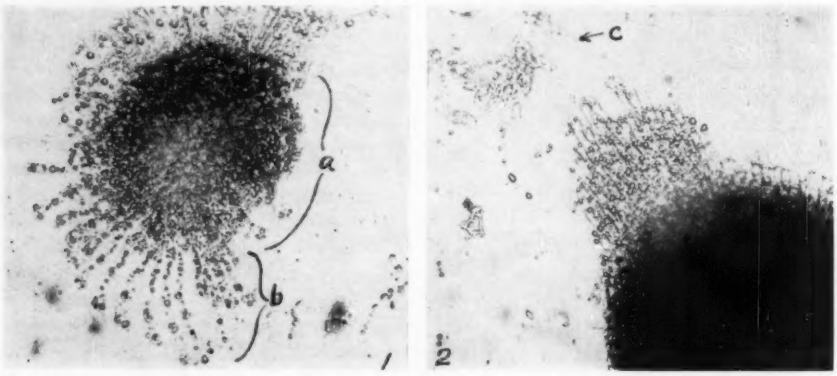
RESEARCH NOTE

IN VITRO SURVIVAL OF GERMINAL CORD TISSUE OF *ASCARIS LUMBRICOIDES* VAR. *SUUM*.

Although the literature concerning in vitro cultivation of helminths is voluminous, there apparently is no published report regarding cultivation of tissues of parasitic helminths. This report concerns the first successful attempts at cultivating tissues of the large roundworm of swine, *Ascaris lumbricoides* var. *suum*.

All microorganisms were eliminated from adult female ascarids by the method of Epps, Weiner, and Bueding (1950, J. Inf. Dis., **87**: 149–151). Finely divided pieces of tissue (explants) from the uterus, germinal cord (rachis), intestine, and cuticle were washed thrice with warm Ringer's solution, placed in Leighton culture tubes, allowed to slightly dry at 37 C enabling adherence to the glass surface, and carefully covered with 2 ml of one of the following media: (1) 90 percent Eagle's and 10 percent calf serum, (2) 90 percent Eagle's and 10 percent swine serum, (3) 90 percent medium "199" and 10 percent calf serum, (4) 90 percent medium "199" and 10 percent swine serum, and (5) 80 percent Hank's, 10 percent calf serum, and 5 percent water extract of adult ascarids. Penicillin (200 units per ml) and streptomycin (50 micrograms per ml) were added. The pH of each medium was 6.8 to 6.9.

Explants of intestinal, uterine, and cuticular tissue in all of the media became opaque, greatly shriveled, and dropped off the glass surface within 4 to 5 days. Explants of the rachis (most terminal portion of the germinal cord) in either Eagle's or medium "199" and calf serum clung to the glass surface, retained the characteristic darkish color, and survived for 46 to 49 days. Only 6 to 9 changes of media were necessary. On the sixth day of cultivation, cells began growing out over the glass surface (figs. 1 and 2). After 3 to 4 weeks, many of



Ascaris lumbricoides var. *suum*

FIGURE 1. In vitro cultivation of explants of germinal cord tissue; a, original explant after 40 days; b, new "growth" that began on 6th day.

FIGURE 2. Proliferation on 40th day; c, cells that had migrated from the original explant.

the cells had proliferated and migrated from the original explant (fig. 2). Most of the cells were narrower and much longer than in the living ascarid. There was no evidence of cell division.—DAVID J. DORAN, *Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

STUDIES ON THE HOST-PARASITE RELATIONSHIPS TO *SCHISTOSOMA JAPONICUM*. VI. ACQUIRED RESISTANCE IN MICE AND MONKEYS INFECTED WITH THE FORMOSAN AND JAPANESE STRAINS

E. H. Sadun,* A. Yamaki,** S. S. Lin** and J. C. Burke**

Resistance to superinfection with homologous strains of *Schistosoma japonicum* has been reported by several authors (Vogel and Minning, 1949, 1953; Lin et al, 1954; Pesigan et al, 1958; Sadun and Lin, 1959; Lin and Sadun, 1959). Heterologous resistance to superinfection against *S. mansoni* cercariae was observed by Vogel (1949) in two monkeys which had been previously infected with *S. japonicum*. In a preliminary abstract, Hunter and Weinman (1960) reported non-reciprocal acquired resistance between *S. mansoni* and *Schistosomatium douthitti* in mice.

In 1956 Hsü and Hsü reported that the Formosan strain of *S. japonicum* develops for a short period in the viscera of man, but does not reach maturity there and must be regarded as a non-human, zoophilic strain. In recent publications, the susceptibility of mice and of monkeys to four geographic strains was determined (Hsü and Hsü, 1960a, 1960b, 1961) and protection against a subsequent challenge with the heterologous strain in monkeys was reported.

In 1958 studies were initiated at the 406th Medical General Laboratory to determine whether a demonstrable resistance to superinfection with the androphilic strain by previous infections with the zoophilic strain can be obtained and vice versa. In the present paper, the relative degree of resistance to homologous and heterologous infections with the Japanese and Formosan strains of *S. japonicum* was studied.

MATERIALS AND METHODS

Young adult white mice weighing approximately 20 g each and Macaca monkeys (*Macaca irus*) weighing approximately 2 kg each were used as test animals. Cercariae of Japanese and Formosan strains of *S. japonicum* were obtained by crushing infected *Oncomelania nosophora* and *O. formosana* snails collected from endemic areas in Japan and Formosa, respectively. At least 10 infected snails were crushed at one time to assure the presence of both sexes among the infecting cercariae. The cercariae were counted and the animals infected as described elsewhere (Sadun and Walton, 1958; Sadun et al, 1959). Since previous experience had indicated that the infectivity of cercariae decreases a few hours after they escape from the crushed snails, only a limited number of animals were exposed to infection at a time. Furthermore, since the infectivity of cercariae varies with the age of the snails and the time of the year at which the snails are collected (Evans and Stirewalt, 1951), each experimental lot was separately controlled.

Necropsies of mice were performed 25 and 26 days after exposure to the challenging infection. This interval of time allowed the schistosomulae to reach the hepatic portal system and grow enough to be easily recovered. At this time, all the worms from the challenging infection could be readily separated from those of the primary infection by their small size and incomplete development except for the groups in which the interval between infections was 30 days or less. The number of worms recovered at necropsy in relation to the number of cercariae to which experimental and control animals were exposed and the mean length of the worms were used as criteria in determining resistance of the hosts to the schistosomes.

Monkeys were exposed 4 times to cercariae of the Formosan strain and challenged with the Japanese strain. Fecal examinations were conducted at regular intervals throughout the experiment. The presence and number of eggs in the stools were determined by the AMS III concentration technic (Hunter et al, 1948). The perfusion technic (Pan and Hunter, 1951) was used for recovery of the developing schistosomes. After perfusion, the intestine and liver were removed and searched for worms that might not have been obtained by perfusion. The recovered schistosomes were examined under the microscope, counted, sexed, differentiated into worms of primary or secondary infections (when intervals between infections exceeded 30 days), and measured. The length of the worms was determined by using a map measure on tracings magnified 5 times with a photographic enlarger. In statistical analyses the usual formulas for the values of t and P were used to determine significance. It was often necessary to compare the mean number of worms in groups which had received an

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immunizing and a challenging exposure with the sum of the means of the worms recovered from controls which had received the first or the challenging exposure only. In these instances, the largest values of variance obtained in either of the two groups were used in the comparison, thus establishing more critical standards for an analysis of the significance of the observed differences.

RESULTS

Experiment 1 (table I). In this first experiment mice were exposed initially to cercariae from the Japanese or Formosan strains and latter had been challenged with the homologous or heterologous strains.

Experiment 2 (table II). In this experiment the challenging exposure followed the initial exposure by 30 days. Once again, in every group the percent recovery of worms from the superinfected animals was lower than that of their controls. The difference was not significant in the series (A) which had been exposed to the Japanese strain. The statistical validity of the differences observed in the other three groups

TABLE I. *Number of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 10 days after the first exposure (30 cercariae).*

Exper. series	No. of mice	Schistosome strain		Results of examination at necropsy 25–26 days after challenge			Comparisons of worm recoveries			
		First exposure	Challenge	No. worms	Mean No.	Percent recovery	Exp. series	Mean No.	Diff.	p
A	15	Japanese	Japanese	450	30	50.0	A	30	2	0.50
B	15	Japanese	Formosan	358	24	39.8	E + G	32		
C	15	Formosan	Formosan	316	21	35.1	B	24	5	0.06
D	15	Formosan	Japanese	369	25	41.0	E + H	29		
E	10	Japanese	—	141	14	47.0	C	21	10	0.01
F	9	Formosan	—	146	16	54.1	F + H	31		
G	10	—	Japanese	181	18	60.3	D	25	9	0.001
H	10	—	Formosan	150	15	50.0	F + G	34		

challenged ten days later with homologous and heterologous strains. The mice included in this experiment were divided into eight groups (experimental series). Those of three series (A, B, and E) were initially exposed to 30 cercariae each of the Japanese strain; those of three other series (C, D, and F) were exposed to 30 cercariae each of the Formosan strain; those of series G and H were kept as uninfected controls. Ten days later all the mice of series A, D, and G were exposed to 30 cercariae each of the Japanese strain. Those of series B, C, and H were exposed to 30 cercariae each of the Formosan strain. The animals of series E and F were not challenged and were used as controls of the first exposure. The results of examination at necropsy conducted 25 and 26 days after the challenging exposure indicated that in all series lower percentages of worms were recovered in the superinfected animals than in their controls. This difference was not statistically significant in the groups (A and B) initially exposed to the Japanese strain, but was highly significant in the groups (C and D) initially exposed to the Formosan strain regardless of whether the is shown by the value of P in table II. The difference in the percent recovery in one of the two series initially exposed to the Formosan strain (D) is highly significant (P less than 0.001).

Experiment 3 (tables III and IV). In this experiment the time interval between the first and the challenging exposures was 60 days. Preliminary experiments had indicated that a large percent of the mice inoculated with 30 cercariae and challenged with 30 cercariae 60 days later died before the completion of the experiment 85 and 86 days from the first exposure. Therefore, in this and the following experiment it was decided to immunize the mice with 20 cercariae and to challenge them with 30 cercariae each. In all groups the percent recovery of worms from the immunized animals was significantly lower than that of their controls. The difference is highly significant (P less than 0.001) in the two series which were challenged with the homologous strain (A and C, table III).

Measurements of the recovered worms were made and compared. As indicated in table IV, the mean length of worms resulting from the

first exposure varied from 19.6 to 20.6 mm. As was to be expected, there was no significant difference in the mean length of worms recovered from the primary infection in the various series. The mean length of worms from the challenging exposure varied from 6.9 to 15.0 mm. In all series the mean length of worms from the superinfected mice was smaller than that of their controls. These differences are highly significant (P less than 0.001) in all the series except that in which mice were infected with the Japanese and challenged with the Formosan strain (Series B).

Experiment 4 (tables V and VI). This ex-

TABLE II. *Number of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 30 days after exposure (30 cercariae).*

Exper. series	Schistosome strains			Results of examination at necropsy 25–26 days after challenge			Comparisons of worms recoveries			
	No. of mice	First exposure	Challenge	No. worms	Mean No.	Percent recovery	Exp. series	Mean No.	Diff.	p
A	9	Japanese	Japanese	167	19	30.9	A	19	8	= 0.07
B	8	Japanese	Formosan	92	12	29.2	E + G	27		
C	9	Formosan	Formosan	130	14	24.1	B	12	9	< 0.01
D	14	Formosan	Japanese	232	17	27.6	E + H	21		
E	9	Japanese	—	137	15	50.7	C	14	7	< 0.05
F	10	Formosan	—	152	15	50.7	F + H	21		
G	10	—	Japanese	122	12	40.7	D	17	10	< 0.001
H	9	—	Formosan	55	6	20.4	F + G	27		

TABLE III. *Number of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 60 days after the first exposure (20 cercariae).*

Exper. series	No. of mice	Schistosome strain		Results of examination at necropsy 25–26 days after challenge			Comparisons of worms recoveries			
		First exposure	Challenge	No. worms	Mean No.	Percent recovery	Exp. series	Mean No.	Diff.	p
A	14	Japanese	Japanese	166	12	24.0	A	12	7	< 0.001
B	14	Japanese	Formosan	110	8	16.0	E + G	19		
C	14	Formosan	Formosan	107	8	16.0	B	8	6	< 0.01
D	11	Formosan	Japanese	151	14	28.0	E + H	14		
E	9	Japanese	—	49	5	25.0	C	8	7	< 0.001
F	9	Formosan	—	52	6	30.0	F + H	15		
G	11	—	Japanese	154	14	46.7	D	14	6	< 0.01
H	9	—	Formosan	82	9	30.0	F + G	20		

TABLE IV. *Mean Length of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 60 days after the first exposure (20 cercariae).*

Exper. series	Schistosome strain		Mean length of worms in mm		Comparison of lengths in mm						
					Worms of first exp.			Worms of challenging exp.			
	First exposure	Challenge	First exp.	Challenge	Series	Length	Diff.	Series	Length	Diff.	p
A	Japanese	Japanese	19.9	6.9	A	19.9	0.4	A	6.9	7.9	< 0.001
B	Japanese	Formosan	20.6	12.0	E	20.3		G	14.8		
C	Formosan	Formosan	20.2	7.0	B	20.6	0.3	D	8.5	6.3	< 0.001
D	Formosan	Japanese	19.9	8.5	E	20.3		G	14.8		
E	Japanese	—	20.3	—	C	20.2	0.6	B	12.0	3.0	= 0.06
F	Formosan	—	19.6	—	F	19.6		H	15.0		
G	—	Japanese	—	14.8	D	19.9	0.3	C	7.0	8.0	< 0.001
H	—	Formosan	—	15.0	F	19.6		H	15.0		

periment was designed essentially like the previous one except for the fact that the time interval between the first and the challenging exposure was 90 days. Once again, the percent of worms recovered from the superinfected series was smaller than that from their controls. The differences are highly significant (P less than 0.001) only in the group which was exposed and challenged with the Formosan strain (Series C).

Experiment 5 (table VII) This experiment was for testing whether resistance is developed in monkeys to the Japanese strain after exposure to several doses of the Formosan strain. Two monkeys (Nos. 1 and 2) were each exposed to approximately 1700 cercariae of the Formosan strain; one (No. 3) was used as unimmunized control and one (No. 4) was used as control of the Formosan strain. The superinfected animals were first exposed to 100 cercariae of the Formosan strain, then to 600 cercariae 20 days later, again 600 cercariae 10 days later (30 days from the initial exposure) and finally 400 cercariae 60 days later (90 days from the first exposure). Ten days after the fourth exposure (100 days from the first exposure), these monkeys and their non-infected controls were each exposed to 700 cercariae of the Japanese strain. One monkey died of undetermined cause. The results of stool examinations conducted every other day revealed that monkeys were free of eggs until 33 days following the challenging

TABLE V. *Number of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 90 days after the first exposure (20 cercariae).*

Exper. series	No. of mice	Schistosome strain		Results of examination at necropsy 25–26 days after challenge						
		First exposure	Challenge	No. worms	Mean No.	Percent Recovery	Comparison of worm recoveries: Exper. series	Mean No.	Diff.	p
A	13	Japanese	Japanese	170	13	26.0	A	13	3	= 0.07
B	13	Japanese	Formosan	147	11	22.0	E + G	16		
C	12	Formosan	Formosan	82	7	14.0	B	11	3	= 0.07
D	14	Formosan	Japanese	212	15	30.0	E + H	14		
E	6	Japanese	—	23	4	20.0	C	7	9	< 0.001
F	9	Formosan	—	55	6	30.0	F + H	16		
G	10	—	Japanese	115	12	40.0	D	15	3	= 0.07
H	9	—	Formosan	93	10	33.0	F + G	18		

TABLE VI. *Mean length of* S. japonicum *recovered from mice challenged with homologous and heterologous strains, 90 days after the first exposure (20 cercariae).*

Exper. series	Schistosome strain		Mean length of worms in mm		Comparisons of lengths in mm: From first exposure			From the challenging exposure			
	First Exposure	Challenge	First Exposure	Challenge	Series	Length	Diff.	Series	Length	Diff.	p
A	Japanese	Japanese	18.4	8.1	A	18.4	3.2	A	8.1	0.7	= 0.3
B	Japanese	Formosan	16.0	7.0	E	15.2		G	8.8		
C	Formosan	Formosan	18.2	8.4	B	16.0	0.8	D	7.8	1.0	= 0.10
D	Formosan	Japanese	17.7	7.8	E	15.2		G	8.8		
E	Japanese	—	15.2	—	C	18.2	1.1	B	7.0	3.3	< 0.001
F	Formosan	—	17.1	—	F	17.1		H	10.3		
G	—	Japanese	—	8.8	D	17.7	0.6	C	8.4	1.9	= 0.03
H	—	Formosan	—	10.3	F	17.1		H	10.3		

As indicated in table VI, the mean length of worms from the first exposure varied from 15.2 to 18.4 mm. No significant difference in mean length of worms recovered from the primary infection was found in any of the experimental series. The mean length of worms recovered from the challenging exposure varied from 7 to 10.3 mm. In all groups the worms from the superinfected series were shorter than those from their controls; however, the difference was highly significant only in one series (B).

TABLE VII. *Results of examinations in monkeys exposed to Formosan and Japanese strains of* S. japonicum.

Monkey No.	Immunizing exposures: Strain	Doses	Total Cercariae	Challenge: Jap. strain	N E P G* Max.	mean**	Necropsy 95 days after challenge: No. worms	Mean length (mm)
1	Formosan	3	1700	700	(Died 41 days from challenge—cause undetermined)			
2	Formosan	3	1700	700	15	60	10	12.0
3	—	—	—	700	246	29.1	54	12.6
4	Formosan	2	350	—	—	—	3***	1.1

* NEPG = Number of eggs per gram of feces.
** = Mean number of eggs from earliest appearance to necropsy.
*** = Males only.

exposure after which eggs were present in the stools of both monkeys until they were sacrificed, 95 days after the challenging infection; however, the number of eggs in the control was considerably higher than in the immunized monkey. The difference in the mean number of eggs between monkeys No. 2 and No. 3 is highly significant (P less than 0.001). At necropsy, 54 worms were recovered from the control monkey and 10 from the superinfected control. The mean length was 12.0 and 12.6 mm, respectively. The stunted male worms were recovered from the control of the Formosan strain (No. 4).

DISCUSSION

Analysis of the data of the foregoing experiments reveals the presence of an acquired resistance to the homologous and heterologous strains of *S. japonicum* following a previous infection with the Formosan or Japanese strains. This resistance is manifested by the reduction in number and size of the developing worms. No difference in the sex ratio of developing worms was observed in the immunized animals.

The results are strikingly similar to those reported previously with the Japanese strain (Sadun and Lin, 1959).

A greater and more rapidly manifested degree of resistance was observed in mice which had been initially infected with the Formosan strain. In fact, in mice which were challenged only 10 days after the primary infection, significant resistance was observed only among those immunized with the Formosan strain. The significantly lower percentage of worms recovered in the immunized animals as well as the stunting effect cound possibly be attributed to a "physiological crowding" rather than to a true immunological phenomenon. However, the results of previous experiments on the acquired immunity of schistosomiasis (Fujinami, 1917; Vogel and Minning, 1948; Vogel, 1949; Vogel and Minning, 1953; El Gindy, 1950; Kagan, 1952; Sadun and Lin, 1959; Lin and Sadun, 1959; Naimark *et al*, 1960), and those obtained with other helminths (Sadun, 1948, 1949) provide strong additional evidence for the interpretation that the observed increased resistance is due to increased host response to immunological stimuli, and persists for variable lengths of time after spontaneous recovery or successful therapy. Furthermore, "physiological crowding" could not possibly apply to the results observed in the monkey superinfected with the Formosan strain, since this animal had fewer and shorter worms than its control.

In addition to demonstrating that cross protection is possible in animals with the Formosan and Japanese strains, these experiments add evidence in support of the view that the presence of schistosome eggs in the tissues are not necessary for the development of acquired resistance. Mice which had been exposed to the Formosan strain of *S. japonicum* developed significant partial resistance to a challenging infection of either the Formosan or the Japanese strain 10 days later at a time when no eggs could possibly have developed. This conclusion appears all the more warranted when considered in conjunction with the apparent resistance observed in the monkey which had been exposed to repeated doses of the Formosan strain, although the zoophilic strain does not produce eggs in the Rhesus monkey (Hsü and Hsü, 1960). These observations are in accord with the results published by Vogel (1949), El Gindy (1950), and Hsü and Hsü (1961), but are at variance with the conclusion reached by Kagan in experiments with unisexual infections of *S. douthitti* (1952) and with infections following treatment (1953). It should be pointed

out, however, that three stunted males were recovered from a monkey infected with the Formosan strain only, and that it is theoretically possible that a few female worms of the Formosan strain develop in the Rhesus monkey and produce eggs which play a role in the development of resistance. The results of experiments currently carried out in which cercariae sterilized by irradiation are used in the development of acquired resistance should help in resolving these conflicting data.

The results of experiments 3, 4 and 5 indicate that when the second infection was given 60 or 90 days after the primary infection, no significant effect could be observed in the number and size of the worms from the primary infection. These results are consistant with and add support to observations reported with other helminths (Sudun, 1948) and could be due to the fact that in 60 days the worms of the primary infection have already grown to a considerable extent and are free in the mesenteric circulation with less possibility of being stunted or destroyed by the increased host resistance.

SUMMARY

Resistance to superinfection with the Formosan and Japanese strains of *S. japonicum* was acquired by mice previously infected with the homologous and heterologous strains. The resistance which was manifested by a reduction in the number and growth of developing worms from the challenging infection was more marked when the Formosan strain was used for the immunizing infection. The significant resistance observed in mice challenged only 10 days after an initial infection indicates that eggs in the host tissues are not necessary for the development of immunity in schistosomiasis. This observation was supported by indications of resistance in a monkey previously exposed to the Formosan strain which normally does not produce eggs in primates.

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RESEARCH NOTE

THE WHITE IBIS, *EUDOCIMUS ALBUS* (LINN.), HOST FOR THE DIPLOSTOMATID TREMATODE *FIBRICOLA CRATERA* (BARKER AND NOLL, 1915) DUBOIS, 1932, IN LOUISIANA.

A single ibis collected at Sarpy, Louisiana, was found infected with two specimens of *Fibricola cratera*. The recovery of *Fibricola* from a natural infection of an avian host is believed noteworthy in light of the postulate of Dubois and Rausch (1950, Bull Soc. Neuch. Sci. Nat. **73**: 19–50) regarding the biological adaptation of these strigeids to mammalian hosts, and the experimental findings of other investigators.

Dubois (1953, Mem. Soc. Neuch. Sci. Nat. **8**: 1–141) divided the Diplostomatidae into two subfamilies, the Diplostomatinae and the Alariinae, using host specificity as a primary character. Members of the subfamily Diplostomatinae are said to be avian parasites, whereas members of the subfamily Alariinae (which includes the genus *Fibricola*) are said to be exclusively parasitic in mammals. However, Leigh (1954, J. Parasit. **40**: 45) has recovered mature specimens of *Fibricola* from chicks, which at hatching had been exposed to metacercariae. Ulmer (1955, J. Parasit. **41**: 460–466) reported similar results. Both investigators (loc. cit.) noted that chicks older than 1 day were refractory to infection with *Fibricola*. They also observed that newly hatched chicks fed *Fibricola* metacercariae eliminated these worms 10 days after exposure. Hoffman (1955, J. Parasit. **41**: 237) was unable to infect pigeons with *F. cratera*.

The two *F. cratera* specimens from the ibis had well-developed hind bodies and vitellaria. One specimen bore a single egg in the uterus. These worms from the ibis were considerably smaller (0.602 and 0.673 mm long) than mature specimens of *F. cratera* (average length 0.880 mm; range 0.602 to 1.435 mm) recovered from heavily infected mink and raccoons in the same locality. The smaller size and fewer numbers of *F. cratera* recovered from the ibis may be due to suboptimal conditions encountered by these worms in the avian intestine. The stomach and intestine of the ibis contained remains of small lizards and amphibian pelvic girdles. I have found *Fibricola* metacercariae in the hindleg and pelvic musculature of *Hyla cinerea* (Schneider) in Louisiana.

This investigation was supported in part by a research grant (E-3386) from the National Institutes of Health, and was done under the direction of Dr. Franklin Sogandares-Bernal.—RICHARD D. LUMSDEN, *Dept. of Zoology, Tulane University, New Orleans, Louisiana.*

RESEARCH NOTE

EARLY DEVELOPMENT OF AN AVIAN FILARIAL WORM IN THE GNAT, *CULICOIDES CREPUSCULARIS*.

The writer has presented circumstantial evidence that biting gnats of the genus *Culicoides* may be the intermediate hosts for some avian filarial worms (1955, J. Parasit. **41**(2): 209–214; 1961, J. Parasit. **47**(3): 441–444). This report gives more direct evidence supporting that hypothesis.

A starling harboring a natural infection of nocturnally periodic microfilariae was used as bait in a *Culicoides* trap. One trial yielded about 50 engorged *Culicoides crepuscularis*. They were transferred to a cage in the laboratory, where nearly all died during the next 4 days. Two which died in the first 24 hours were examined, and one of these had two microfilariae in the gut contents. Fifteen dead specimens were dissected on the third and fourth days, and the last two survivors were sacrificed 88 and 92 hours after engorgement. Five of these 17 insects contained either one or two developing worms; some may have been present in other hosts, but rendered unrecognizable by post-mortem changes. The small number of worms per host probably is a consequence of the minute amount of blood which such small insects can ingest.

Bacterial action had disrupted internal organization in worms from the dead insects, but not enough to conceal the fact that there were some differences between three-day and four-day worms. The living parasites recovered from the last two hosts dissected showed a prominent subterminal anal opening on a large protuberance; a small opening, presumably excretory or reproductive, in the anterior third of the body; and evidence of a pseudocoel forming. The worms were lying motionless in thoracic muscles, with no sign of host reaction to their presence. They were distinctly larger than microfilariae, but comparative measurements have not been made.

Two criticisms are possible of the assumption that these worms originated in the starling and were developing in a normal host. They could have been acquired from another host species in a blood meal prior to the one taken from the starling; and they might not have grown to the infective stage had the hosts lived.

It seems unlikely that one-third of randomly trapped gnats would harbor filarial worms, all in the same stage of development. Also, the parasites were immature enough to suggest that they could not have been in the host since an earlier blood meal was taken. The obvious normality of the living worms, and the absence of host reactions, argue that they would have developed to the infective stage had the *Culicoides* survived several more days.

Later development was not followed because of unsolved problems in maintaining the hosts in laboratory cages. *Culicoides* appear only seasonally, in late spring and early summer, so work on these infection experiments cannot be continued for nearly a year. Hence, this fragmentary report is made now for the information of others interested in the subject.

Grateful acknowledgement is made of the assistance in trapping *Culicoides* rendered by Mr. John Ackermann and Mr. Robert Workman, as National Science Foundation Undergraduate Research Participants. This research was supported by grant E-2583 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.—EDWIN J. ROBINSON, JR., *Department of Biology, Kenyon College, Gambier, Ohio.*

SEROLOGY OF MARINE TREMATODES. I. THE ACTION OF ANTISERA AGAINST STRIGEOID AND SCHISTOSOME CERCARIAE*

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Most of what is known about the serology of trematode cercariae concerns schistosomes which are of economic importance to man. Except for the work of Vogel and Minning (1949b) and Hendricks and Cort (1956), serological studies of the effect of immune sera on non-mammalian schistosome cercariae have been neglected. Moreover, there have been no investigations along this line involving marine trematodes. The phenomena previously described for mammalian schistosome cercariae include cercarial precipitation (Papirmeister and Bang, 1948), agglutination (Liu and Bang, 1950), and the Cercarienhüllenreaktion (CHR) (Vogel and Minning, 1949a, b; Standen, 1952; Kagan, 1955; Stirewalt and Evans, 1955; Kagan and Levine, 1956; and Hendricks and Cort, 1956). The present work was undertaken, therefore, to supplement our knowledge concerning serological reactions of trematodes in general, especially marine forms which naturally infect the hornshell snail, *Cerithidea californica*.

Cerithidea californica, a mudflat snail, is found abundantly along the estuaries and back bays of the southern California coast. This marine snail serves as a favorable host for more than 20 species of trematodes (Martin, 1955). The effect of normal sera of a number of vertebrates upon certain cercarial types naturally infecting *C. californica* has been reported (LeFlore, 1960). It was found that reactions of these cercariae in normal vertebrate sera are quite similar to those reported by Culbertson (1936), Culbertson and Talbot (1935), Tubangui and Masilungan (1936), Standen (1952), Stirewalt and Evans (1955), and Kagan and Levine (1956).

The present study is the first of a series to determine the in vitro reaction patterns of antisera against the life-cycle stages of representative marine trematodes which naturally infect *Cerithidea californica*. Three species of trematode cercariae were used in these experiments: two species of strigeoids, a small unidentified cercaria and a large form, *Mesostephanus appendiculatus* (Martin, 1961), and avian schistosome cercariae.

MATERIALS AND METHODS

Antisera were produced in chickens by injecting cercarial antigen of the small strigeoid cercariae. The reaction of the small strigeoid cercariae and of the larger cercariae of *Mesostephanus appendiculatus* was studied in these antisera. The avian schistosome cercariae were studied in antisera against *Schistosoma mansoni*.

Small strigeoid cercariae, the cercariae of *Mesostephanus appendiculatus*, and avian schistosome cercariae were collected from naturally infected *Cerithidea californica*. The snails were collected from the mudflats of small ponds from Upper Newport Bay, Huntington Beach, and Seal Beach, California. The snails were washed and placed in finger bowls with a small amount of sea water, usually two snails to a bowl. Snails were isolated when the desired cercarial types were observed in the sea water. Careful examination of the emerged cercariae was made before collection to determine that only one cercarial species was present. The small strigeoid cercariae were concentrated, lyophilized under vacuum, sealed in vials, and stored in the deep freezer at −20 C following the techniques of Pellegrino and Nunes (1956). The antigen was prepared prior to each inoculation by homogenizing 2 mg of lyophilized small strigeoid cercariae in 2 ml of phosphate-buffered saline (0.85 percent NaCl, pH 7.2) and suspended in Fruend's adjuvant (Fruend and Bonanto, 1944). The mixture was emulsified with a mortar and pestle for several minutes.

Twelve chickens were divided into four groups of three each. Group I received six intraperitoneal injections 3 times a week on alternate days for 2 weeks. The inocula consisted of the antigen (2 mg of lyophilized cercariae in 2 ml of phosphate-buffered saline) suspended in 1.5 ml of paraffin oil and 1.0 ml of Aquaphor (a lanolin-like substance) per inoculum. Group II received six intraperitoneal injections on alternate days for 2 weeks

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of the same amount of the antigen suspended in the same amount of paraffin oil and Aquaphor with 0.1 mg of heat-killed (1 hour at 70 C), lyophilized *Mycobacterium phlei* per inoculum. Groups III and IV were control groups. Group III received injections of 2 ml of phosphate-buffered saline suspended in the adjuvant per inoculum as in group I; whereas, group IV received injections of 2 ml of phosphate-buffered saline suspended in the adjuvant per inoculum as in group II. Blood was withdrawn 3 to 4 weeks after the last injection by cardiac puncture, allowed to clot, ringed, and centrifuged. The sera were collected and stored in the deep freezer without preservatives until ready for use. Both experimental and control sera were used in ring precipitation tests by overlaying the sera with a 0.1 percent phosphate-buffered saline (0.85 percent NaCl, pH 7.2) extract of lyophilized cercariae. The reaction of small strigeoid cercariae and the cercariae of *Mesostephanus appendiculatus* was studied in unheated and heated (56 C for 0.5 hour) antisera by placing a drop of living cercariae in a depression slide with three to four drops of sera and observing under a microscope at intervals for 24 hours. Controls were prepared with normal sera and filtered sea water and observed for the same length of time. Heated and unheated double dilutions of antisera were tested against both species of cercariae.

Schistosomes from *Cerithidea californica* were tested in serum from two Puerto Rican patients with chronic schistosomiasis mansoni. The two sera were obtained from Dr. I. G. Kagan. The cercariae were collected with a bacteriological loop from the surface film of the sea water containing infected snails, and placed in depression slides containing three to four drops of heated or unheated dilutions of the sera. Normal heated or unheated human sera and filtered sea water were used as controls. Observations were made at room temperature for a period of 24 hours.

RESULTS

The sera from chickens of Groups I and II were positive for antibodies against small strigeoids as evidenced by positive precipitin ring tests. When tested against living cercariae, the antisera from chickens of group II reacted almost immediately and at higher dilutions than the antisera from chickens of group I. For this reason, the description of the sequence of events will be restricted to antisera of the former group. In control sera, no change was observed in the morphology of the cercariae except an occasional dropping of the tail from the body.

Effect of homologous antisera on small strigeoid cercariae. Certain definite and immediate morphological changes were observed in small strigeoid cercariae when immersed in unheated, undiluted, homologous antisera. Within the first 5 minutes of contact with the antisera, the cercariae secreted large masses of mucoid sticky material from the oral glands. The cuticle of the tail and body became thickened and wrinkled. Within 10 minutes a thick, clear, transparent membrane appeared first around the tail, followed by its appearance around the body. The tail and body sheaths became thicker and appeared complete after 15 minutes (fig. 1). The cercariae adhered to one another upon contact. Throughout the first 20 minutes, large masses of cercariae became agglutinated. Attachment of the cercariae occurred in the oral regions (fig. 2), and appeared to be due to the sticky mucoid material secreted at their oral ends. Motility was impaired and slow; however, it was observed throughout a 24-hour period.

In 1:8 dilutions of antisera the cercariae reacted in a similar manner to those observed in undiluted antisera. In 1:32 and 1:64 dilutions of antisera, however, the beginning of reaction was delayed so that the first signs of change in the cercariae occurred after 1 hour of contact with the antisera. As in undiluted antisera, the cercariae reacted first by producing large amounts of mucoid material at the openings of the oral gland ducts, followed by a thickening and wrinkling of the cuticle. The sheaths which appeared involved only the body of the cercariae. Although the tail was not involved in sheath formation, it became wrinkled and the furci were curled. The reaction was considered complete after 1.5 hours of contact with the antisera. Agglutination occurred in the same manner as in the lower dilutions of antisera. Cercariae in a 1:256 dilution of antisera were normal in appearance and were comparable to those of the controls. Inactivation of the small strigeoid cercarial antisera at 56 C for 30 minutes only delayed the time of the reaction of the cercariae. The first effects were seen after 15 minutes, after which the reaction proceeded as in unheated antisera.

The cercariae of *Mesostephanus appendiculatus* studied in small strigeoid antisera reacted as did the small strigeoids, except that the beginning of the reaction was delayed approximately 15 minutes. The sheaths formed around the body of the cercariae were somewhat thinner and a sheath was formed around the tail. The cercariae were agglutinated in smaller masses by the mucoid material secreted by the oral glands.

Effect of Schistosoma mansoni *antisera against avian schistosome cercariae from* Cerithidea californica. Schistosome cercariae from *C. californica* in undiluted *S. mansoni* antisera which had been inactivated at 56 C for 30 minutes remained normal for the first 30 minutes. Then the oral glands began to secrete large amounts of sticky mucoid material which caused the cercariae to agglutinate in small clumps of three to four cercariae. This material was seen to adhere to the cercariae and after 24 hours of observation large amounts of it were still present in the oral region. After the first hour in the antisera the cuticle appeared wrinkled and thickened, first in the midbody region and then anteriorly and posteriorly to involve the rest of the body and tail. Also the furci curled (fig. 4). Observations after 4, 6, and 12 hours of contact with the antisera revealed no change of this condition. The cercariae were still alive as evidenced by slow movements. No enveloping membrane or sheath characteristic of the CHR was observed throughout this period. In dilutions higher than 1:4 the cuticle of the cercariae was normal and only a small amount of the sticky material was exuded.

Schistosome cercariae in fresh and heat-inactivated, normal human sera remained normal and motile for 24 hours (fig. 3). The only detrimental effect observed was an occasional dropping of the tail from the body. This may have been due to injury to the cercariae when transferring them to the serum medium.

DISCUSSION

The in vitro response of small strigeoid cercariae from *Cerithidea californica* to homologous antisera produced in chickens was characterized by agglutination and CHR. The changes which occurred in the cercariae included the production of large amounts of sticky secretions from the oral glands, followed by morphological cuticular changes and the formation of a rigid and compact CHR membrane which ensheathed the tail and head region of the cercariae. Although the CHR has been clearly defined in schistosome cercariae, it has not been demonstrated in other species of trematode cercariae.

There are many similarities between the response of strigeoid cercariae and schistosome cercariae in antisera; the differences, however, are minor. This similarity in serological response to immune sera may be due to a phylogenetic relationship between the strigeoids and the schistosomes. According to the classification of La Rue (1957), the order Strigeatoidea is comprised of four suborders and includes all families of the Digenea which have fork-tailed cercariae. The suborder Strigeata comprises three superfamilies and includes the superfamily Strigeoidea containing the families Strigeidae and Cyathocotylidae, and the superfamily Schistosomatoidae containing the family Schistosomatidae.

The sequence of events in CHR described in the present report is similar to that described by Vogel and Minning (1949a) and Stirewalt and Evans (1955) for mammalian schistosome cercariae. In the reaction described by these investigators, the sheath did not always involve the tails of the cercariae. Stirewalt and Evans (1955) reported envelopment of the tail only in strongly reacting sera. Envelopment of the tail first in strigeoid cercariae is in agreement with the results of Hendricks and Cort (1956) with avian schistosomes. These workers, however, did not observe secretions of large amounts of sticky mucoid material in the oral region prior to sheath formation. In higher dilutions of antisera, membranes were formed only around the body of the strigeoid cercariae; the tail was not involved.

Agglutination of strigeoid cercariae was concurrent with sheath formation and occurred in diluted as well as undiluted antisera. Scattered clumps of fully ensheathed strigeoid cercariae were observed throughout the surrounding medium of antisera. The fact that many of the cercariae adhered to parts of the bodies of other cercariae by the oral region suggested that the sticky mucoid material was responsible for this agglutination. Agglutination of schistosome cercariae without the formation of pericercarial membranes has been reported by Liu and Bang (1950). A complete analysis of the agglutination phenomenon by Stirewalt and Evans (1955) revealed that agglutination of these cercariae did not occur without the formation of the pericercarial membrane and was attributed to the stickiness of the oral material. Hendricks and Cort (1956) suggested that agglutination results if the envelope is not formed immediately over the anterior end of the cercariae. Since profuse amounts of this oral precipitate were initially produced by the strigeoid

cercariae in undiluted as well as diluted antisera, agglutination resulted first and was followed by sheath formation beginning in the area of the tail and spreading rapidly anteriorly to the body region where the cercariae were already attached at their oral ends.

The fact that cross reactions were obtained with the large strigeoid cercariae of *Mesostephanus appendiculatus* in small strigeoid antisera indicated that this reaction is not specific. Although the species of the small strigeoid cercariae is unknown, it is highly probable that these cercariae are closely related species and are similar in antigenicity. This finding is in accord with Vogel and Minning (1949b), Meleny and Moore (1954), and Hendricks and Cort (1956) who obtained cross reactions between schistosome species.

Furthermore, it may be stated that the antibodies for the CHR of strigeoid cercariae is heat stable, since heat treatment of the antisera only slightly delayed the onset of the reaction.

The reaction of avian schistosome cercariae from *Cerithidea californica* in *Schistosoma mansoni* antisera was similar to that reported by Hendricks and Cort (1956) for avian schistosomes observed in high dilutions of homologous antisera. The reactions, characterized by the secretion of large amounts of mucoid material anteriorly, agglutination, and morphological cuticular changes, but no CHR, are considered as weak antigen-antibody reactions, suggesting that these cercariae have some similarity in antigenicity.

SUMMARY

Antisera produced in chickens by injection of a lyophilized cercarial antigen of small strigeoid cercariae from the mudflat snail, *Cerithidea californica*, immediately produced a reaction which was characteristic of the CHR when tested in vitro against such cercariae. These antisera when tested against the cercariae of *Mesostephanus appendiculatus*, a large strigeoid cercariae from *C. californica*, produced a reaction identical to that of the small strigeoid cercariae in homologous antisera. It it suggested that this cross reaction indicates a similarity in antigenicity as well as a close relationship between these species.

Avian schistosome cercariae from *C. californica* were studied in *Schistosoma mansoni* human antisera. The cercariae produced oral sticky secretions which caused them to adhere to one another, indicating an antigen-antibody reaction, but there was no CHR.

ACKNOWLEDGMENTS

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PLATE I

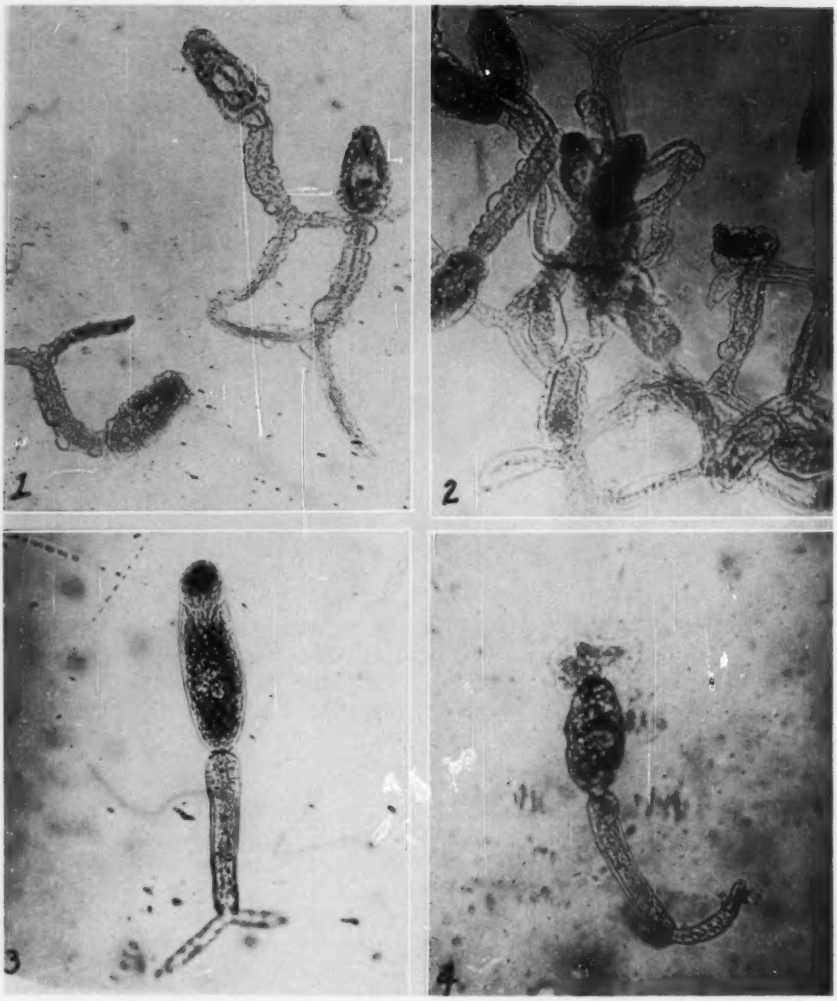

FIGURE 1. CHR of small strigeoid cercariae in homologous antisera.

FIGURE 2. Agglutination and CHR of small strigeoid cercariae in undiluted homologous antisera.

FIGURE 3. Avian schistosome cercaria from *Cerithidea californica* in normal inactivated human serum.

FIGURE 4. Avian schistosome cercaria in *Schistosoma mansoni* antisera.

PASSAGE OF EGGS BY HOSTS INFECTED WITH *SCHISTOSOMA MANSONI*, WITH EMPHASIS ON RODENTS*

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Successful propagation of the schistosomes and other trematodes is dependent upon satisfactory host-parasite relationships and the passage of viable eggs. In the case of schistosomiasis there is a direct relationship between egg-laying capacity of the parasite and pathologic conditions developing in the definitive host. Although general recognition has been given to the importance of egg laying by the schistosomes, whether in man or in lower mammals, there seems to be little information on the actual passage of eggs by infected hosts. Since the production of eggs by the schistosomes may be used as a criterion in determining effects of intrinsic (immunity, etc) and extrinsic (drugs, etc) factors upon the welfare of the parasite, for host-parasite compatibilities and as an indication of possible reservoir host potentials, this phase of schistosomiasis has been followed as an aspect of our biological studies.

This paper presents a study of the passage of eggs by several species of laboratory and wild mammals infected with *Schistosoma mansoni* in the NAMRU-3 laboratory at Cairo, United Arab Republic.

MATERIALS AND METHODS

After evaluation of various mammals (mostly rodents) (Kuntz, 1955) for their susceptibility to infection by *S. mansoni*, several species, including mediocre as well as good hosts, were chosen for this study. Egg passage patterns were observed for the following rodents: Albino mice; house mice (*Mus musculus*), house rats (*Rattus rattus*), Nile rats (*Arvicanthis nilotica*), spiny back mice (*Acomys cahirinis*), gerbils (*Gerbillus pyramidum*), and bandicoots (*Nesokia indica*).

Limited observations were made also on several other mammals with known low grades of susceptibility to infection with *S. mansoni*.

All hosts were exposed to 200 cercariae of *S. mansoni* since previous studies had shown this to be an optimal parasite load for small and medium size hosts. Cercariae were obtained from *Biomphalaria* dipped from the irrigation canals in the vicinity of Cairo, and cercariae from 10 to 20 snails were pooled prior to exposure of hosts. All hosts were exposed to cercariae which had emerged from the same stock of infected snails. Hosts were partly immersed in warm water for 15 to 20 minutes prior to exposure to encourage the passage of urine and feces before the animals were placed in the cercarial suspension.

Egg counts were based upon an examination of feces passed during a period of 24 hours. After maceration of pellets in slightly hypertonic saline the suspension was passed through a 60-mesh wire screen to remove the coarse debris. The remainder was prepared for examination after sedimenting for a few minutes between each of four to six washings, the final one with aerated fresh water. One-fourth of the final sediment was examined on a microslide by low magnification of a compound microscope. The total number of eggs was computed by multiplying the number of viable and non-viable eggs by four. Viability of eggs was judged by motility and the general condition of the miracidium or egg contents.

Some of the hosts were sacrificed after a period of several weeks if no eggs appeared in the feces. Hosts were examined at this time or at the time of death due to infection, to make observations on the presence of eggs in various organs and to obtain adult schistosomes.

RESULTS AND DISCUSSION

As shown in a companion study of the egg passage by various hosts infected with *Schistosoma haematobium* (Kuntz, 1961), there was marked variation in the intervals between time of exposure and appearance of first eggs in feces, and in the duration of infection before death of animals infected with *S. mansoni* (table I). Consequently, data cannot be satisfactorily grouped for each host species. Although 10 to 15 individuals of each of the

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smaller host species were infected, pertinent data are given for only a few individuals which serve to illustrate patterns of egg passage and allied host-parasite relationships.

Stool samples were examined from other host species in which it was realized that egg passage would be nil or minimal. These data have not been recorded in table I. Previous susceptibility studies (Kuntz, 1955) had demonstrated that goats, guinea pigs, cats, hedghogs (*Hemiechinus auritus*), and albino rats and rabbits were poor hosts for Egyptian *S. mansoni*, but a few eggs were observed in the walls of intestines of some goats, cats, albino rats and hedgehogs. In the present investigation only limited numbers of nonviable eggs were passed by 1 guinea pig, and 1 cat, and a few viable as well as nonviable eggs were found in fecal samples from 2 hedgehogs. In such animals there was an obvious low grade susceptibility to infection. Infections frequently terminated spontaneously and schistosomes, when present, were few in number and poorly developed. Observations at autopsy revealed that some schistosomes deposited viable eggs in the walls of the intestine but the host-parasite conditions apparently were not favorable for the final release of eggs from tissues into the lumen.

As indicated in table I, rodents are moderately acceptable hosts for *S. mansoni*. Susceptibility and egg laying patterns, however, varied considerably from one species to another, as well as between individuals of the same host species. Eggs first appeared in the feces 5 to 9 weeks after infection, regardless of definitive host type. There was great variation in the numbers of eggs passed, in the percentages of viable eggs, in the duration of peaks of production and in the number of schistosomes recovered at autopsy. In most cases, irrespective of host species or the numbers of eggs involved, egg counts were irregular but the patterns of egg passage had a tendency to follow the course of a normal curve. Usually, after the first detection of eggs, there were limited number of eggs in pellets during the first 4 to 8 days followed by rather

TABLE I. *Eggs of* Schistosoma mansoni *passed with feces of infected rodents.*

Host	Duration of egg production (weeks)†	Peak of egg production (weeks)†	Number of eggs per day		Percentage of eggs viable	Worms present at postmortem examination†††			
			Range	Mean††		M	F	Pairs	Total
Albino mice									
AM-1	24	10–14	16–432	154	44	2	7	–	9
AM-2	20	13–17	32–460	203	47	–	4	3	10
AM-3*	40	4–6	0–188	56	49	1	2	–	3
AM-4	26	3–5	0–212	106	39	2	1	4	11
AM-5*	17	7–12	80–614	461	53	10	1	2	15
House mice—*Mus musculus*									
MM-1	22	3–5	0–68	31	16	2	2	–	4
MM-2*	22	5–7	0–84	36	26	–	3	–	3
MM-3	11	4–6	4–88	55	67	2	–	3	8
House rats—*Rattus rattus*									
RR-1	15	3–4	0–88	26	28	2	–	–	2
RR-2	15	3–5	0–104	36	31	–	1	–	1
Nile rats—*Arvicanthis niloticus*									
AN-1*	53	2–5	40–1268	473	55	4	5	8	25
AN-2	53	6–8	56–484	208	49	2	4	5	16
Spiny back mice—*Acomys cahirinus*									
AC-1	60	4–8	4–360	156	49	2	4	4	14
AC-2	60	4–8	0–184	105	66	–	3	5	13
AC-3*	60	6–10	0–336	108	47	5	3	4	16
Gerbils—*Gerbillus pyramidum*									
GP-1	28	11–13	8–320	122	43	2	3	4	13
GP-2*	28	9–13	48–304	144	21	6	3	5	19
GP-3	20	2–4	56–376	173	36	3	1	4	12
Bandicoots—*Nesokia indica*									
NI-1*	11	3–5	18–404	269	54	1	2	8	19
NI-2*	8	5–7	504–1164	794	41	3	24	3	33
NI-3*	7	5–7	268–384	333	49	–	13	41	95

† Weeks after detection of eggs in feces.
†† Eggs/day (mean) computed on total number of eggs/number of examinations.
††† Each host exposed to 200 cercariae.
* Hosts died due to infection, injury, etc.

sharp increases the following 2 to 4 weeks when a plateau was established. The peak of egg production as well as the duration of this plateau were individual characteristics with no explanation for the wide ranges recorded. Subsequently eggs were passed in decreasing quantities until death or until hosts were sacrificed.

The peaks of egg production for the better hosts (*Arvicanthis* and *Nesokia*) were of moderate duration, the numbers of eggs were fairly consistent and counts during the plateau stage were only slightly less than those in the period of peak production. In these hosts, and especially in *Arvicanthis,* the reduction of egg passage was very gradual, suggesting a balance or tolerance between host and parasite which probably accounted for the overall passage of greater quantities of eggs. In poor hosts (*Mus* and *Rattus*), by contrast, the peaks of egg production occurred during the first 3 to 5 weeks and were followed by irregular plateaus and sharp decreases in egg passage with negative records on many examinations.

There was a wide range in the interval between the peak of egg production and the time at which definite egg reduction occurred among the different host species. In albino mice, *Gerbillus,* and *Acomys* (acceptable hosts) reductions did not become marked until late in the course of infection, i.e., 14 to 26 weeks. After this period there were days when the records were negative, followed by erratic appearances of both viable and nonviable eggs. The latter became more numerous after the 20th week. Pellets of *Arvicanthis* contained eggs upon each examination for 39 to 45 weeks and there were moderate numbers of eggs until the time of sacrifice at 53d week. Increase in the percentage of nonviable eggs became marked after the 30th to 40th weeks. On the contrary, in house mice and house rats negative stools occurred as early as the 2nd to 4th weeks and on occasion only nonviable eggs were detected during this period. Subsequent to the arbitrary period of peak of production the counts never equalled those during the first 2 weeks of egg passage.

Most of the acceptable hosts had varying quantities of viable and nonviable eggs deposited in the liver as well as at different locations along the intestinal tract. Eggs, however, were more common in the small than in the large intestine and the proportion of nonviable eggs increased at all sites of deposit with prolonged infection. Marked involvement, i.e., lesions and areas of hemorrhage, occurred in the small intestine of some animals which were passing large quantities of eggs, semi-diarrheic feces and blood at the time of death. There was no obvious correlation between the sites of deposition and the numbers of eggs passed.

An evaluation of host-parasite and host-egg-release relationships indicated that the proportion of viable to nonviable eggs was variable and to some extent, a matter of chance, depending upon the nature of lesions and tissue responses in individual hosts. Overall counts were erratic throughout the periods of observation but there was a definite increase in the percentage of nonviable eggs for all hosts as infections continued beyond the period of peak egg production. There was no constant relationship between the numbers of eggs passed and the quantity of pellets produced by hosts during the 24 hour periods of collection.

The passage of nonviable eggs by experimentally infected animals has frequently been employed as an indication of incompatibility of host and parasite. In the present and in previous susceptibility studies it is obvious that schistosomes were unable to attain normal maturity in some hosts (albino rats, dogs, other carnivores, etc), and eggs, if deposited in host tissues were present in limited quantities. Observations of animals infected with the Egyptian *S. mansoni* have indicated, however, that the majority of eggs passed by normal, mature female parasites in an acceptable host appear to be viable. Admittedly, exceptions occur in young females before normal egg rhythm is established, when there is an absence of spermatozoa or there is malfunction of the reproductive organs. It is believed that the passage of nonviable eggs by an animal is to be expected and, when present, is an indication that eggs have been retained in host tissues until rendered nonviable by age or by "foreign body" and protective (immunologic) reactions. Thus man (theoretical, optimal host), as well as lower vertebrates which may be considered as less desirable hosts, pass both viable and nonviable eggs. Recently deposited eggs escaping from intestinal lesions are likely to be viable, whereas those erupting from a site where eggs have been tissue trapped stand a greater chance of being nonviable.

Other investigators, e.g., Faust et al (1934) employing monkeys with the Puerto Rican *S.*

mansoni, Vogel (1958) using monkeys infected with *S. japonicum*, and Moore and Sandground (1956) studying hamsters as hosts for Puerto Rican *S. mansoni* and *S. japonicum*, have observed patterns of egg passage similar to those described in the present report. Faust et al stated that the decrease of egg passage subsequent to the acute stage of infection was conceivably due to a slowing down of the egg-producing mechanism of the parasite, but greater weight was given to the fact that egg reduction resulted from tissue reactions in the bowel. Moore and Sandground (1956) studying the egg production capacity of schistosomes in hamsters found large quantities of eggs deposited in different tissues and emphasized the importance of the percentage of eggs that are trapped and never escape. The latter authors estimated that only 22 and 16 percent, respectively, of all eggs produced by *S. mansoni* and *S. japonicum* were passed in the feces.

Earlier studies (Kuntz, 1961) with *S. haematobium* had shown that an efficient egg passer (*Arvicanthis*) produced greater quantities of pellets and eggs during the early morning hours. Similar observations were made for egg passing activities of *Arvicanthis* and *Nesokia* infected with *S. mansoni*. In most instances, greater numbers of eggs were passed with the increased volume of feces from 0400 to 0800. The overall increase was similar to that recorded for *Arvicanthis* infected with *S. haematobium* but less consistent. It is assumed that the greater egg production during these hours may have been due to accelerated activities characteristic of nocturnal animals.

As a rule the hosts with the greater number of parasites passed larger quantities of eggs. However, this relationship did not always hold and on several occasions good hosts (*Arvicanthis*) with only a few schistosomes passed numerous eggs indicating, perhaps, that satisfactory egg release activities allowed the passage of a large proportion of eggs deposited within the intestinal walls. Although all animals listed in table I possessed schistosomes at time of sacrifice there were others in the series which were negative, but passed eggs irregularly until a few days before autopsy. Apparently tissue-trapped eggs were slowly and irregularly released into the intestine for several weeks after spontaneous termination of the infection.

In experimental schistosomiasis it has been assumed that pathologic involvement, immunologic responses, infection, and patterns of egg passage, etc., in suitable lower vertebrates are somewhat comparable and analogous to similar conditions found in man. Susceptibility studies have stressed the importance of egg-laying abilities of the parasite and the passage of eggs by hosts other than man. In evaluating host-parasite relationships, Martins (1958) has listed the presence of viable and nonviable eggs in organs, feces, and urine as one of the basic criteria in appraising reservoir-host potentials. In spite of the recognized importance of eggs and host-egg-passage relationships it seems that this facet in our understanding of experimental schistosomiasis has been neglected. The present study has emphasized the fact that the proportion of viable to nonviable eggs and the erratic quantities of eggs passed by different hosts is probably a reflection of egg-release phenomena at the site of deposition in intestinal tissues. Furthermore, it is believed that in an evaluation of the influences of extrinsic factors (supra-infections, introduction of drugs, dietary deficiencies, etc) upon infection, recognition should be given to the fact that nonviable eggs probably should be considered as normal constituents of stools passed by schistosome infected animals.

SUMMARY

The egg-passage pattern of Egyptian *Schistosoma mansoni* has been followed in a series of wild and laboratory mammals representing hosts with different grades of susceptibility and thus varying host-parasite relationships. Egg counts have shown that the passage of eggs irrespective of hosts have a tendency, quantitively, to follow the path of a normal curve. Irregularities in this curve, deviation in the proportion of viable to nonviable eggs, and termination of egg passage differs with the host species and may even be an individual characteristic. Examinations of many hosts over a period of months indicate that the passage of nonviable schistosome eggs is probably a normal situation resulting from a release of aged and tissue-bound eggs by the host.

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IN MEMORIAM

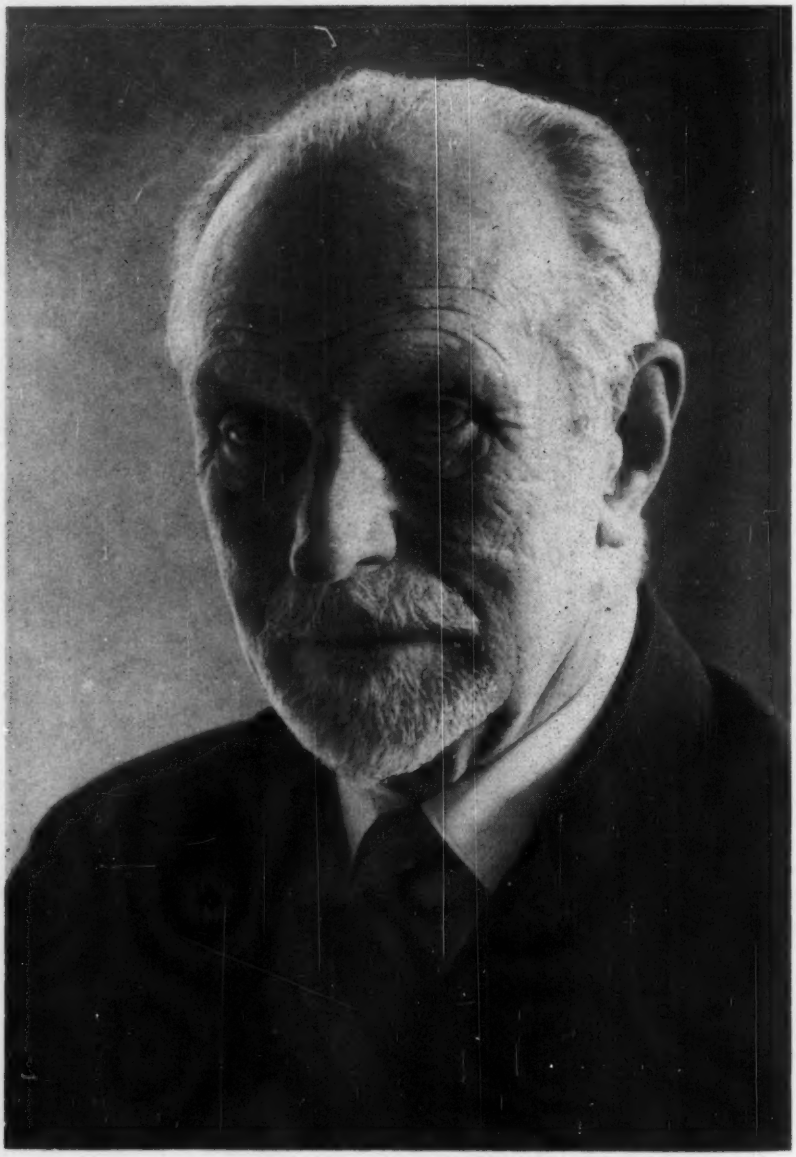
Erich Martini (1880–1960)

Erich Martini M.D., Ph.D., Sc.D. Hon., Professor-Emeritus in the medical faculty and Vorsteher of the department of entomology in the Institut für Schiffs-und Tropenkrankheiten in Hamburg, since 1932 a Foreign Honorary Member of the American Society of Parasitologists, died December 2, 1960. He was born March 19, 1880, in Rostock, Schwerin, where his father was Oberlandesgerichtspräsident, and where he made his Abitur in 1899. Subsequently, he studied at the universities of Rostock, München and Tübingen. His training was broad and thorough, and led to doctorates in both medicine and philosophy. From the tutelage of Barfurth, Blochmann, Richard Hertwig, Seeliger, and others he developed into a sincere, sedulous student of minute anatomy, especially of the invertebrate animals, with a deep interest and penetrating insight into the philosophy of biology. In 1906 he began as Assistent at Rostock and later became Prosektor an der Anatomie and Privatdozent; in 1909 he transferred to Tübingen. His early publications were concerned with the morphology, ontogeny, and systematics of nematodes, rotifers, and appendiculate tunicates. He was impressed by the determinate cleavage of animals with mosaic development, the extent of cell-constancy among invertebrates, and its biological significance. His insight into biological phenomena is illustrated by two early papers (1909): "Darwinismus und Zellkonstanz," and "Über Eutalie und Neotenie."

Although he had not published on insects, in 1912 he was called to Hamburg to organize, install and direct a department of entomology in the Institut für Schiffs-und Tropenkrankheiten. In the 33 years that he led the entomologischen Abteilung, it became the foremost laboratory of medical entomology in Germany and one of the leading centers of the world. Since insects have cell-constancy in somatic tissues, his transfer of interest to higher invertebrates was a natural one. Furthermore, since insects so often transmit diseases, the new position at Hamburg permitted a fruitful combination of medicine and biology. His earliest entomological studies were on mosquitos and malaria and the epidemiology of the disease. Later work was concerned with the role of insects in the spread of other parasitic diseases. With incisive analysis, he attempted to evaluate the factors involved in the origin and development of parasitism; the relations between hosts, parasites and vectors; between features of physical geography, climate and the spread of insect-transmitted infections. The inherent biological principles were set forth in his "Wege der Seuchen" (1936) and amplified in "Seuchen im Menschen" (1959), written in his 78th year; a treatise on theoretical and biological principles as they pertain to epidemiology of insect-transmitted disease. His "Lehrbuch der medizinischen Entomologie" (1923, 4th ed. 1952) is probably the most accurate, complete and outstanding treatise on the subject in any language. After official retirement in 1945, he continued his work with unabated vigor, exerting a beneficial influence and inspiration in Germany and abroad. The author of more than 300 technical articles, he also wrote on occasion for newspapers and did a delightful biography of Prof. Dr. Bernhard Nocht, the founder and first Director of the Tropeninstitut.

A tall, personable man, of ruddy complexion, a vigorous, diligent and talented craftsman, a strict disciplinarian who could not tolerate sloth, sham or mediocrity, Professor Martini was at the same time a kind and generous person, with friends and admirers in many lands. An active member of scientific societies, he received high honors. He was president of the VIIth International Congress of Entomology (1938) and some of the finest tributes were paid on the occasions of his 75th and 80th birthdays. On his 75th birthday, Heft I, 1955 of the Zeitschrift für Angewandte Zoologie carried the following headline: "Dieses Heft ist dem

hervorragenden Forscher und Lehrer Prof. Dr. med. et phil. Erich Martini, Hamburg, gewidmet. Die Autoren der einzelnen Beiträge gehören dem grossen Kreis seiner Schüler und Freunde an." Celebrations were arranged by the Tropeninstitut and other groups. On his 80th



birthday, at a special ceremony, the University of Hamburg conferred the honorary degree, Doctor of Science, on its distinguished member. In his passing, the medical and biological world has suffered a grievous loss.

The American Society of Parasitologists extends sympathy to his widow, his daughter and his son.—HORACE W. STUNKARD.

SELLACOTYLE VITELLOSA, A NEW TROGLOTREMATID TREMATODE FROM THE MINK IN LOUISIANA*

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During studies of trematodes encysted in Louisiana crayfishes, several mink from infected crayfish localities have been examined for parasites. The present report is of 12 small trematodes collected from a young mink shot in a swamp behind the Valentine Sugar Plantation near Valentine, Louisiana. This same mink was heavily infected with a species of *Fibricola*.

All measurements in the following description are in millimeters.

Sellacotyle vitellosa sp. n.
Figures 1 to 3

Host: *Mustela vison vulgivaga* (Bangs), family Mustelidae

Location: Small intestine

Locality: Swamp behind Valentine Sugar Plantation, near Valentine Louisiana

Holotype: U.S. National Museum Helminthological Collection No. 38919

Diagnosis (measurements on five of twelve specimens): *Sellacotyle.* Body pyriform, 0.36 to 0.42 long by 0.22 to 0.24 wide. Forebody 0.14 to 0.17 long. Cuticle completely spined. Oral sucker subterminal, 0.09 to 0.10 long by 0.09 to 0.10 wide. Acetabulum equatorial, without processes, 0.10 to 0.12 long by 0.12 to 0.14 wide. Sucker width ratio 1:1.13 to 1.37. Pre-pharynx very short, almost appearing absent. Pharynx 0.03 to 0.04 long by 0.03 to 0.04 wide. Esophagus about same length as pharynx, usually extending from pharynx to anterior border of acetabulum. Ceca two, one on each side of body; extending from posterior end of esophagus to posterior border of acetabulum.

Genital pore median, immediately postacetabular; followed by a short, shallow, genital atrium. Testes two, posterior to or slightly overlapping posterior border of acetabulum, one on each side of body; dextral testis 0.12 to 0.14 long

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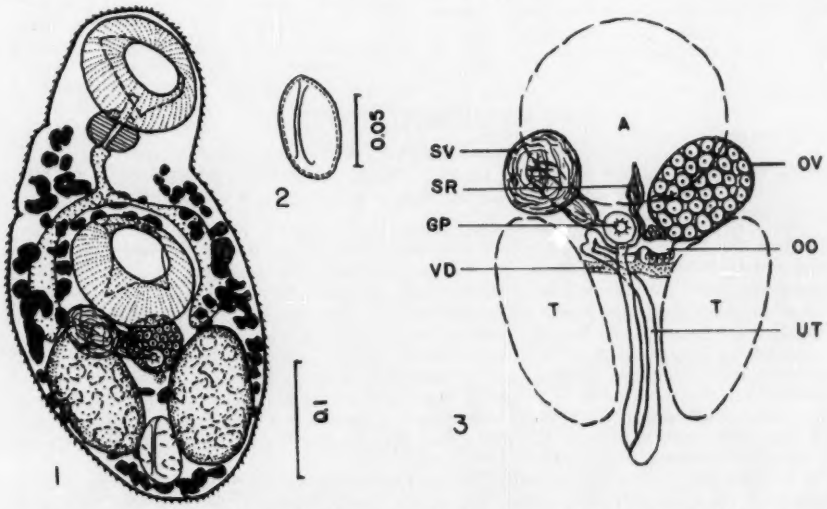

FIGURE 1. *S. Vitellosa*, ventral view of whole mount. Vitellaria dorsal to testes are dashed-in.

FIGURE 2. Same, collapsed egg.

FIGURE 3. Same, composite sketch of genitalia. Acetabulum and testes dashed-in to show relationship to other organs; unless otherwise specified, all figures drawn with the aid of a Leitz camera lucida. The projected scales are in millimeters. Abbreviations used: A, acetabulum; GP, genital pore; OO, ootype; OV, ovary; SR, seminal receptacle; SV, seminal vesicle; T, testis; UT, uterus; VD, vitelline duct.

by 0.08 to 0.10 wide; sinistral testis 0.13 to 0.14 long by 0.06 to 0.09 wide. Seminal vesicle bipartite, usually extending transversely or obliquely from between anterior end of dextral testis and acetabulum, sometimes overlapping either or both organs, to a short pars prostatica which inserts into genital atrium. Ovary usually overlapping posterior border of acetabulum and in contact with anterior border of sinistral testis, sometimes mesial, overlapping posterior border of acetabulum; roundish or elliptical in shape, 0.03 to 0.04 long by 0.04 to 0.06 wide. Oötype immediately posterior to ovary. Seminal receptacle usually mesial, overlapping posterior border of acetabulum. Laurer's canal not observed. Uterus completely intertesticular, extending transversely towards dextral testis from oötype, descending almost to posterior end of body, ascending directly to genital atrium. Vitelline follicles large, irregularly shaped, extending posteriorly on each side of body from level of pharynx, coalescing dorsally in region of cecal bifurcation, sometimes overlapping anterior border of acetabulum dorsally, overlapping ceca dorsally and ventrally on each side of body, intruding into hindbody to overlap testes dorsally and fill posttesticular space. Vitelline duct sometimes swollen to form a receptacle dorsal and level or slightly posterior to oötype between testes. Collapsed eggs 0.06 to 0.07 long by 0.04 wide. Excretory vesicle not clearly observed in stained material, appearing sac-like and extending from excretory pore at posterior end of body to between posterior borders of testes in living material.

DISCUSSION

Sellacotyle vitellosa closely resembles *S. mustelae* Wallace, 1935, from which it differs by its more posterior and extensive vitelline distribution, very reduced pars prostatica and lack of acetabular processes. Wallace (1935) named and described *Sellacotyle mustelae* from mink, probably *Mustela vision letifera* Hollister and *M. vison lacustris* (Preble), in Minnesota and Ontario. He also succeeded in experimentally infecting rats, mink, dogs, cats, ferrets, foxes, racoons, and skunks by feeding fish infected with metacercariae of *S. mustelae*.

REFERENCE

WALLACE, F. G. 1935 A morphological and biological study of the trematode *Sellacotyle mustelae* n.g., n.sp. J. Parasit. 21: 143–164.

RESEARCH NOTE

RAILLIETIELLA (*HEYMONSIA*) *HEMIDACTYLI* HETT, 1934, FROM *HEMIDACTYLUS MOBOUIA* IN PUERTO RICO, WITH A CORRECTION OF THE IDENTITY OF *R. HEBITIHAMATA* SELF AND KUNTZ, 1961.

Several specimens of a pentastomid taken from a gecko, *Hemidactylus mobouia*, collected at Rio Piedras, P. R., have been determined as *Raillietiella* (*Heymonsia*) *hemidactyli* described by Hett in 1934 from *H. gleadovi* and *Calotes versicolor* collected in Burma (Hett, 1934, Proc. Zool. Soc. Lond., July 1934, pt. 2, pp. 425–431). Since this species of *Raillietiella* is not recorded from the Western Hemisphere and since H. *mobouia* was introduced from Africa, it can be presumed that the parasite was probably introduced with the host.

Little is known about the life cycles of the various recognized species of *Raillietiella*. However, since this genus occurs in a large number of host species which live on vegetation or small invertebrates, it must be presumed that their life cycles are not at all like those of the porocephalids which pass through mammals and reptiles in completing their life cycles. In the case of the species reported here it appears that the parasite has also found the requisites of its life cycle in the West Indies.

In 1960 Self and Kuntz described *R. hebitihamata* from *H. frenatus* taken on Lan Yü Island, off the coast of Formosa (Self and Kuntz, 1961, J. Parasit. 46: 885–888). The senior author who prepared the description inadvertantly overlooked the description of *R.* (*Heymonsia*) *hemidactyli* by Hett, to which it conforms in almost every respect. *R. hebitihamata*, Self and Kuntz, must, therefore, be considered as synonymous with Hett's specimens.—J. TEAGUE SELF, *University of Oklahoma*, AND JULIO GARCIA-DIAZ, *University of Puerto Rico.*

THE HELMINTH FAUNA OF NICARAGUA. II. OBSERVATIONS ON *PARALLOPHARYNX ARCTUS* CABALLERO, 1946

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Division of Biological Research, Alaska Department of Fish and Game, Juneau

Caballero (1946) described a new genus and species of the family Plagiorchiidae for the reception of a single specimen of a fluke taken from a Guatemalan iguanid. In 1954 the author had the opportunity to collect a variety of helminths in eastern Nicaragua among which was another specimen of this apparently rare genus and species. At the present time our knowledge of the genus *Parallopharynx Caballero* (loc. cit.) is still limited to the original description of the type and only specimen. Accordingly, it appears worthwhile to present a brief description of the author's Nicaraguan specimen, also taken from an iguanid.

MATERIALS AND METHODS

The fluke was removed while still active from the intestine of the lizard and fixed under light coverslip pressure with F.A.A. solution. It was subsequently stained with Semichon's acetocarmine and mounted in balsam. All measurements are given in millimeters.

I wish gratefully to acknowledge the help of Dr. Thomas R. Howell, Department of Zoology, University of California at Los Angeles, who provided the opportunity for the author to accompany him on one of his field trips to Nicaragua. The field work was supported in part by a grant from the University of California Association of Tropical Biogeography to Dr. Howell. Dr. Howell's assistant, Mr. John Zoeger, was particularly helpful in collecting the present material.

OBSERVATIONS

Parallopharynx arctus Caballero, 1946 (Fig. 1)

Description: Body elongate, 13.36 long by 1.12 broad, bluntly pointed at each end. Cuticular spines lacking. Oral sucker subterminal, broadly oval, 0.29 long by 0.27 wide. Acetabulum approximately round, 0.24 in diameter, located 2.38 from anterior end. Prepharynx present, 0.27 long by 0.10 wide. Pharynx broadly oval, 0.26 long by 0.21 wide. Esophagus very short poorly demarcated. Intestinal caeca unequal in length, left caecum reaching within 0.24 of posterior end, other caecum 0.66 shorter.

Testes tandem, oval, located intracaecally and dorsally in posterior third of body; anterior testis 0.39 long by 0.32 wide; posterior testis 0.42 long by 0.33 wide. Cirrus pouch well developed, 1.52 long by 0.20 wide, extending parallel to and slightly to left of midline from a point 0.94 behind posterior margin of acetabulum to genital pore located 0.35 in front of anterior margin of acetabulum and slightly to left of midline on ventral surface. Seminal vesicle and pars prostatica well developed. Ovary oval, located just postequatorial, 0.40 long by 0.30 wide. Uterus transversely folded, intracaecal, extending posteriorly to end of shorter intestinal caecum, anteriorly to posterior end of cirrus pouch, ventral to other reproductive organs. Metraterm weakly developed. Vitellaria in groups of oval follicles, four groups on left side extending well past posterior testis, three groups on right side not reaching level of posterior testis, extending anteriorly on both sides to a point 1.62 from posterior margin of acetabulum primarily extracaecal with medial borders overlapping caeca ventrally and dorsally. Eggs oval and operculated, 0.21 long by 0.16 wide. Excretory pore terminal, bladder sacciform with primary lateral collecting ducts reaching anteriorly almost to pharynx.

Host: Iguanid lizard (sp. ?).

Habitat: Intestine.

Locality: El Recreo, 10 miles west of Rama Zelaya, Nicaragua.

Specimen: In author's collection.

DISCUSSION

The present specimen, though generally somewhat larger, agrees in all essential features with Caballero's Guatemalan type specimen. Accordingly, modification of the generic or specific concept is not required.

LITERATURE CITED

CABALLERO Y C., E. 1946 Estudios helmintologicos d la region onchocercosa de Mexico y de la republica de Guatemala. Trematoda. II. Presencia de Paragonimus en reservoios naturales y description de un nuevo genero. An. Inst. Biol. **17**: 137–165.

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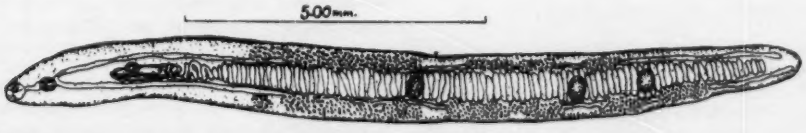

FIGURE 1 *Parallopharynx arctus* Caballero, 1946. Ventral view of adult.

CRYPTOGONIMIDAE (DIGENEA: TREMATODA) FROM PHILIPPINE FOOD FISHES

CARMEN C. VELASQUEZ

Zoology Discipline, University of the Philippines

The present paper deals with digenetic trematodes belonging to the family Cryptogonimidae Ciurea, 1933, collected from Philippine food fishes.

To date *Neochasmus microvatus,* (Tubangui, 1928) Tubangui et Masilungan, 1944, is the only known Philippine representative. The topotype is herein described and reported for the second time in the same host species, *Glossogobius giurus* Buchanan-Hamilton with an additional locality record.

Three species previously reported by Yamaguti, namely *Paracryptogonimus acanthostomus* Yamaguti, 1934, from Japan and Celebes (1934, 1952), *Metadena apharei* (Yamaguti, 1942) Yamaguti, 1953, from Okinawa (Yamaguti, 1942) and *Pseudomentadena celebesensis* Yamaguti, 1952, from Celebes ((Yamaguti, 1942) 1952) are here recorded and described from Philippine waters for the first time in similar, if not from the same host species. One new species of *Paracryptogonimus* Yamaguti, 1934 and one new genus are proposed, and a new combination is made.

The writer wishes to express her gratitude to Dr. George R. LaRue, Animal Parasite Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Maryland, for his valuable suggestions and criticisms and to Mr. Agustin Umali, Ichthyologist, Philippine National Museum for the identification of the fish hosts.

Unless otherwise stated the same methods of collection and staining as given in my previous papers were used. All measurements are expressed in millimeters unless otherwise indicated; drawings were made with the aid of a camera lucida.

Metadena apharei (Yamaguti, 1942) Yamaguti, 1953.

Syn. *Siphoderina apharei* Yamaguti, 1942.

(Figure 7)

Host: *Lutianus* sp.

Location: Intestine.

Locality: Malabon, Rizal (open sea), Luzon Island, Philippines.

Prevalence: 4 specimens in one host examined.

Topotypes: U. S. Natl. Mus. Helm. Coll. No. 39080.

Description (measurements based on 3 specimens): Length 1.78 to 2.79; width 0.69 to 0.85; oral sucker 0.12 to 0.14 by 0.16 to 0.23; prepharynx 0.046 to 0.069 long; pharynx 0.05 to 0.09 by 0.09 to 0.12; esophagus 0.07 to 0.12 long; intestinal ceca extending to posterior extremity; acetabulum 0.09 to 0.12 in diameter; anterior testis 0.3 to 0.44 by 0.35 to 0.41; posterior testis 0.27 to 0.37 by 0.29 to 0.46; vesicula seminalis 0.12 to 0.16 in maximum width; ovary 0.21 to 0.35 in diameter; receptaculum seminis 0.09 to 0.15 in maximum width. Eggs operculate, 15 to 20 by 7.5 to 9.5 microns.

The present material although relatively smaller in size agrees with Yamaguti's (1942) description of *Metadena apharei* (Yamaguti, 1942) Yamaguti, 1953, in all details except in the presence of circumoral spines measuring 23 by 5.8 microns, occurring in a single row. The spines (not figured) were seen in only one specimen which was crushed accidentally. They can be easily overlooked because of their small size or they may be lost due to post mortem changes. Ten spines were counted around half the circumference of the oral sucker, others probably missing.

In addition to complete morphological agreement with Yamaguti's species including the relative egg size, the hosts of both his and my specimens belong to the same family Lutianidae.

Price (1940) listed *Stegopa* Linton, 1910, as a synonym of *Metadena* Linton, 1910; then Manter (1947) considered *Siphoderina* Manter, 1934, to be a synonym of *Metadena* and presented a generic diagnosis; consequently *Siphoderina apharei* Yamaguti, 1942, became *Metadena apharei* (Yamaguti, 1942) Yamaguti, 1953.

Manter (1940) states (p. 415): "Mueller and Van Cleave consider the oral spines a subfamily distinction between Heterophyinae and Neochasminae. Since these spines are absent or present in marine trematodes as closely related

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to each other as *Siphoderina* and *Paracryptogonimus*, they do not seem to constitute more than a generic difference."

Metadena differs from the closely related genus *Paracryptogonimus* Yamaguti, 1934, chiefly in the lack of circumoral spines, the nature of the vitellaria, character of the lobulated ovary and the coarsely lobed testes.

In view of the presence of circumoral spines in the present material, which Yamaguti perhaps failed to see, *Metadena apharei* (Yamaguti, 1942) Yamaguti, 1953, should be *Paracryptogonimus apharei* (Yamaguti, 1953) comb. n.

Pseudallacanthochasmus grandispinus gen. n., sp. n.
Figure 5

Host: *Lutianus* sp.

Location: Small intestine.

Locality: Malabon, Rizal (open sea), Luzon Island, Philippines.

Prevalence: 5 gravid specimens and one immature in one host.

Type and paratype: U.S. Natl. Mus. Helm. Coll. No. 39079.

Specific diagnosis (based on 2 whole gravid, 3 partially damaged specimens): Body elongate, sub-cylindrical, spinose, 2.26 to 3.45 long; 0.23 to 0.33 wide; broad anteriorly, rounded posteriorly (in type). Larval eye spots hardly discernible. Oral sucker terminal, funnel shaped, 0.09 to 0.18 by 0.12 to 0.2, with wide aperture, ventral margin marked with shallow incisions; circumoral spines relatively large, in single row of 23 to 25 (type), measuring 30 to 33 long by 7.5 to 10 microns at base. Prepharynx 0.1 to 0.23 long (4 specimens), expanded at anterior gradually tapering towards pharynx; pharynx comparatively small, muscular 0.05 to 0.12 by 0.06 to 0.12; esophagus short 0.05 to 0.09, bifurcating slightly anterior to anterior extent of vitellaria; ceca simple, reaching nearly to posterior extremity. Acetabulum feeble, 0.12 to 0.14 in diameter, embedded in parenchyma near junction of first and second thirds of body. Testes 0.25 to 0.35 by 0.14 to 0.19 (4 specimens), longitudinally elongate, slightly indented at periphery (type), extra-cecal and intercecal, tandem, dorsal, near posterior extremity of body. Seminal vesicle 0.07 to 0.11 in maximum width, cylindrical, voluminous, winding between ovary, seminal receptacle and acetabulum; pars prostatica cylindrical. Ductus ejaculatorius opening into genital pore located in ventro-genital fold at acetabulum towards right (in type). Ovary slightly lobed, 0.07 to 0.12 in diameter, situated just below receptaculum seminis, near middle of body; germiduct arising from anterior part of ovary, joined by seminal receptacle, Laurer's canal and vitelline reservoir. Vitellaria consisting of fine, delicate follicles confluent at level of esophageal bifurcation, in acetabulo-ovarian zone dorsal and lateral to ceca producing an interlacing effect. Uterus composed of descending and ascending limbs, descending limb describing coils dorsally and between testes to posterior extremity, coils of ascending limb more or less following similar path, discharging into genito-acetabular pouch. Eggs, oval, light brown, 15 to 17 by 6 to 10 microns. Excretory pore terminal, excretory vesicle Y-shaped; stem long, moderately wide (in immature specimen) bifurcating posterior to acetabulum but anterior to ovary, arms reaching to posterior margin of anterior sucker.

The present worm bears a very close resemblance to *Allacanthochasmus* Van Cleave, 1933, but differs fundamentally in the shape and relative size of oral sucker, number of circumoral spines, length and character of the prepharynx, arrangement and nature of the vitellaria, position of the testes, anterior extent of the uterus and excretory arms. The host belongs to the family Lutianidae.

A new genus *Pseudallacanthochasmus* is therefore proposed with the following diagnosis. This genus should be placed near *Allacanthochasmus*.

Pseudallacanthochasmus gen. n.

Generic diagnosis: Cryptogonimidae. Body small, elongate, spinose. Eye spots present. Oral sucker funnel-shaped, terminal with single row of spines. Prepharynx and pharynx present. Esophagus short. Ceca terminating at posterior extremity. Acetabulum feeble, smaller than oral sucker, embedded in body wall, forming acetabulo-genital complex at its anterior margin. Testes tandem, proximate, situated near posterior extremity. Vesicula seminalis relatively wide, convoluted, anterior end opening into genital atrium at genito-acetabular pouch. No cirrus pouch proper. Ovary slightly lobate, median to submedian, ventral, post-acetabular. Receptaculum seminis and Laurer's canal present. Vitellaria follicular, acinous, extending along ceca in lateral fields from intestinal bifurcation to ovary, confluent meso-dorsally producing interlacing effect. Uterine coils passing between testes and reaching nearly to posterior extremity. Eggs small, operculate, dark brown to light brown. Excretory vesicle Y-shaped, bifurcating just posterior to acetabulum but anterior to ovary, arms reaching posterior margin of oral sucker. Intestinal parasite of marine fishes.

Type species: Pseudallacanthochasmus grandispinus.

Neochasmus microvatus (Tubangui, 1928)
Tubangui and Masilungan, 1944

Syn. *Metadena microvata* Tubangui, 1928
Metadena ovata Tubangui and Francisco, 1931
(Figures 3 and 4)

Host: *Glossogobius giurus* Buchanan-Hamilton.

Location: Stomach (few) and small intestine.

Locality: Pasig, Alabang, Cardona, Binan-

gonan, Rizal Province; Sukat, Cavite Province; Sta. Rosa and Laguna de Bay, Los Baños, Laguna Province; all in Luzon Island, Philippines.

Topotypes: U. S. Nat. Mus. Helm. Coll. No. 39078 also in Zoology Discipline, University of the Philippines' Helm. Coll. Nos. 368 (1)f-394 (1)f; 484 (1)f; 733(2)e.

Prevalence: Several thousand in 63 hosts out of 73 examined.

Diagnosis (measurements based on 10 gravid specimens): Body 0.29 to 0.67 by 0.14 to 0.34; oral sucker 0.069 to 0.115 by 0.069 to 0.13; circumoral spines 3.5 by 1 micron, 18 counted in one specimen; pharynx (5 specimens) 0.023 to 0.04 long; esophagus (3 specimens) 0.028 to 0.035 long; acetabulum 0.035 to 0.064 in diameter; ovary trilobate, immediately anterior to testes and toward left of body; testes symmetrical, 0.046 to 0.115 by 0.046 to 0.13. Eggs operculate, yellowish brown 16 to 20 by 10 to 14 microns. Excretory vesicle Y-shaped, main stem bifurcating just posterior to testes, arms reaching almost to oral sucker.

The above measurements together with the general morphology fall within the range of variations of Tubangui's (1928) specimens and those of Tubangui and Masilungan (1944). It is now being reported as *Neochasmus microvatus* (Tubangui, 1928) Tubangui and Masilungan, 1944, in the same host *Glossogobius giurus* Buchanan-Hamilton, from different localities in Luzon Island.

Tubangui (1928) described *Metadena microvata* and later Tubangui and Francisco (1930) reported it as *Metadena ovata*. In 1944 Tubangui and Masilungan based on careful study of the type specimen and more material, placed the parasite in the genus *Neochasmus* Van Cleave and Mueller, 1932; consequently *Metadena microvata* and *Metadena ovata* are synonyms of *Neochasmus microvatus* (Tubangui, 1928), Tubangui and Masilungan, 1944. Yamaguti (1953, 1958) without noting the synonymy listed *Metadena microvata* and *Neochasmus microvatus* as separate species.

Paracryptogonimus acanthostomus Yamaguti, 1934
Figure 6

Host: *Lutianus* sp.

Location: Small intestine.

Locality: Malabon, Rizal (open sea), Luzon Island.

Topotype: U. S. Natl. Mus. Helm. Coll. No. 39077; also in Zoology Discipline, University of the Philippines Helm. Coll. No. 730a.

Prevalence: 13 in one host examined.

The worms were fixed in FAA solution under coverslip and stained with Paracarmine at 4:1 dilution.

Description (measurements from 10 specimens): Body 1.36 to 2.32 by 0.46 to 0.81; oral sucker 0.092 to 0.12 by 0.12 to 0.21; coronet of 32 to 34 oral spines, 20 by 5 microns (spines not figured); prepharynx 0.023 to 0.05; pharynx 0.05 to 0.09 by 0.07 to 0.12 (9 specimens); esophagus 0.023 to 0.07 (8 specimens); acetabulum 0.07 to 0.12 by 0.09 to 0.14; anterior testis 0.21 to 0.37 by 0.16 to 0.25, posterior testis 0.25 to 0.42 by 0.16 to 0.28; vesicula seminalis 0.09 to 0.16 in maximum diameter; ovary 0.23 to 0.35 in diameter; receptaculum seminis 0.09 to 0.17 maximum width. Eggs 15 to 20 by 8 to 10 microns.

The present worm is referred to *Paracryptogonimus acanthostomus* Yamaguti, 1934. It agrees not only in general anatomy and relative body measurements, but also the host, *Lutianus* sp.

Full accounts of this species were given by Yamaguti (1934) from specimens collected from Japan and from Macassar, Celebes, in 1953. Variability in the shape and arrangement of the testes was noted in the Philippine material but is attributed to fixation procedures.

Paracryptogonimus manilensis n. sp.
Figure 1

Host: *Lutianus vitta* (Quoy and Gaimard) (type host). *Parachaetodon ocellatus* (Cuvier and Valenciennes).

Location: Intestine.

Locality: Manila Bay, Philippines (type locality), Malabon, Rizal, Luzon Island, Philippines (bought from Central Market, Manila, Philippines).

Prevalence: 3 in 3 hosts (*Lutianus vitta*) examined; one in 4 hosts (*Parachaetodon ocellatus*) examined.

Type: U. S. Nat. Mus. Helm. Coll. No. 39076.

Paratypes: Zoology Discipline, University of the Philippines, Helm. Coll. Nos. 526(2)f, 541 (1)f, 542(2)f, 570(1)f.

Diagnosis (based on 4 fairly extended gravid specimens): Body elongate, flattened, rounded posteriorly, 1.52 to 1.64 long, 0.44 to 0.53 wide at level of testes. Cuticle minutely spined, hardly discernible. Pear-shaped dermal gland cells numerous at anterior part of body. Larval eye spots present. Oral sucker terminal 0.09 to 0.12 by 0.1 to 0.13. Circumoral spines present (not figured), number not determined due to post mortem changes. Prepharynx 0.023 to 0.12 long. Pharynx 0.046 to 0.048 by 0.046 to 0.58. Esophagus 0.023 to 0.035. Ceca terminating near posterior extremity along side of excretory vesicle. Acetabulum 0.044 to 0.067 in diameter, embedded in circular fold of body wall near anterior fourth of body. Testes elongate, bean-shaped, diagonal, partly extra-cecal; anterior testis 0.28 to 0.35 by 0.16 to 0.18; posterior testis 0.28 to 0.44 by 0.18. Genital pore immediately anterior to acetabulum; vesicula seminalis extending from genital pore posterad as far as ovary, inflated, twisted in terminal portion. Cirrus or prostatic complex absent. Ovary 0.09 to

0.18 in maximum diameter, ventral, at mid-body, coarsely lobate, lobes irregularly radiating from common center. Seminal receptacle present, partly covered by anterior lobules of ovary. Laurer's canal present. Vitelline reservoir dorsal and mesad to ovary. Vitelline follicles acinous, in bunches on each side of body, dorsal and lateral to intestine extending anteriorly to acetabulum, almost to bifurcation of esophagus and posteriorly to level of testes. Descending limb of uterus extending posteriad between testes, describing several coils between testes and posterior extremity, then ascending limb passing between testes, describing coils anterior to left testis, and finally discharging at genital pore. Eggs small, oval, dark brown, 14.5 to 16 by 7 to 9.5 microns. Excretory vesicle Y-shaped, pore terminal; stem rather wide, dorsal, median, bifurcating immediately posterior to ovary; arms fairly wide at level of intestinal bifurcation, reaching to pharynx.

Type species: *Paracryptogonimus manilensis.*

Type host: *Lutianus vitta* (Quoy and Gaimard).

Type locality: Manila Bay, Manila, Philippines.

This worm is closely related to *Paracryptogonimnus acanthostomus* Yamaguti, 1934, but differs essentially in the more anterior extent of the vitellaria, the relative size and shape of testes, more coarsely lobate ovary and more posterior extent of the uterus.

Pseudometadena celebesensis Yamaguti, 1952
(Figures 2 and 8)

Host: *Lates calcarifer* (Bloch).

Location: Stomach, small intestine and intestinal ceca.

Locality: Malabon, Rizal, Luzon Island.

Topotypes: U. S. Natl. Mus. Helm. Coll. No. 39075; also in Zoology Discipline, University of the Philippines Helm. Coll. No. 91a, 92a, 451(1)f, f2, 576(1)d, f, 735e.

Prevalence: Over 500 specimens in 12 of 14 hosts examined.

Description (measurements based on 10 gravid specimens): Body 0.43 to 1.17 long, 0.25 to 0.74 in maximum width; oral sucker 0.056 to 0.115 in diameter; spines of oral coronet 0.012 long (not figured); prepharynx 0.03 to 0.064; pharynx 0.012 to 0.023 in diameter; esophagus 0.07 to 0.12; acetabulum 0.06 to 0.18 by 0.05 to 0.12; testes 0.02 to 0.18 by 0.023 to 0.12. Anterior part of vesicula seminalis 0.023 to 0.13, posterior portion 0.04 to 0.32 in maximum width. Ovary 0.023 to 0.13 by 0.035 to 0.12.

From the general anatomy and body measurements the present specimens belong to *Pseudometadena celebesensis* Yamaguti, 1952. The species is here reported for the first time in the Philippines in the same host, *Lates calcarifer*.

The nature of the excretory vesicle which Yamaguti (1952) failed to see in his material is shown in figure 8. The Y-shaped excretory vesicle divides near the middle of the body, the arms reaching to the pharynx.

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EXPLANATION OF PLATE I

FIGURE 1. Ventral view of *Paracryptogonimus manilensis* sp. n. from *Lutianus vitta*.

FIGURE 2. Ventral view of *Pseudometadena celebesensis* Yamaguti, 1952.

FIGURE 3. *Neochasmus microvatus* (Tubangui, 1928) Tubangui et Masilungan, 1944, showing the circumoral spines and the Y-shaped excretory vesicle.

FIGURE 4. *Neochasmus microvatus.* Ventral view.

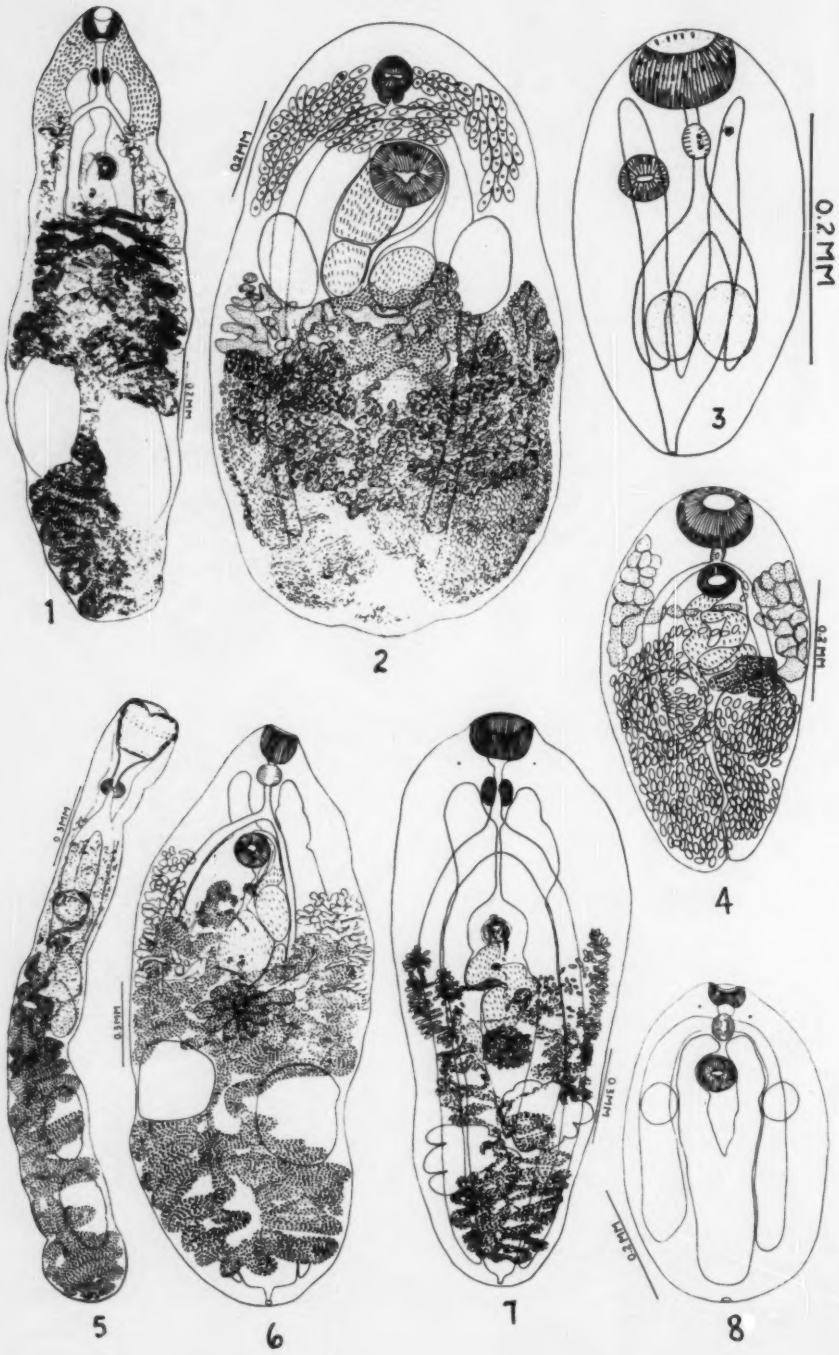
FIGURE 5. *Pseudallacanthochasmus grandispinus* gen. n., sp. n. Ventral view.

FIGURE 6. *Paracryptogonimus acanthostomus* Yamaguti, 1934. Ventral view.

FIGURE 7. *Paracryptogonimus apharei* (Yamaguti, 1942), comb. n. Ventral view.

FIGURE 8. *Pseudometadena celebesensis* Yamaguti, 1952, showing the Y-shaped excretory vesicle.

PLATE I



SIX VIRGULATE XIPHIDIOCERCARIAE FROM OREGON, INCLUDING REDESCRIPTIONS OF *ALLASSOGONOPORUS VESPERTILIONIS* AND *ACANTHATRIUM OREGONENSE*

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The life histories of *Allassogonoporus vespertilionis* Macy and *Acanthatrium oregonense* Macy, two lecithodendriid trematodes of bats, were determined by Knight and Pratt (1955). Included in their study were descriptions of the virgulate xiphidiocercariae for each parasite. Both cercariae were shown to develop into metacercariae in larval caddis flies.

While investigating host-parasite relationships between these cercariae and their arthropod hosts, I collected snails reported by the above authors to harbor the larval stages of these two trematodes. *Fluminicola virens* (Lea), the snail host for *A. vespertilionis*, shed three species of virgulate cercariae, all superficially similar in appearance. From *Oxytrema silicula* (Gould), the snail host for *A. oregonense*, emerged five species of virgulate cercariae, three of which were very similar. Knight and Pratt's descriptions of *A. vespertilionis* and *A. oregonense* cercariae were found to be inaccurate and insufficiently detailed to allow one to distinguish their cercariae from the other virgulate cercariae that emerged from these snails. The cercariae of *A. vespertilionis* and *A. oregonense* are, therefore, redescribed in this paper. Included also are descriptions of four new virgulate xiphidiocercariae which emerged from the prosobranch snail, *Oxytrema silicula*.

METHODS AND MATERIALS

Snails were collected from Shot Pouch Creek, near Burnt Woods, Lincoln County, Oregon. They were placed in dishes containing stream water and left overnight at room temperature. Infected snails were segregated according to the type of cercaria shed by them and kept for future use in aerated aquaria at 60 C.

Most of the techniques used here were patterned after those of Hall (1959). Newly emerged cercariae were studied alive and after fixation in hot 3 percent formalin. Gross morphological features and spatial relationships of parts were obtained from studies of many partially extended living cercariae under light cover-glass pressure. Distortion of the virgula and changes in the appearance of penetration glands were noted in cercariae which had been under minimal cover-glass pressure from 10 to 15 minutes and in specimens stained intravitally with neutral red. Consequently, new slide mounts of freshly emerged, unstained cercariae were prepared frequently while determining the morphology of living specimens. Details of the penetration glands and excretory systems were obtained by quickly compressing living cercariae with a cover-glass. As noted by Hall (1959), flame cells were more active and hence more readily seen when cercariae were mounted in amphibian Ringer's solution instead of water. All measurements are given in millimeters. The body measurements of living cercariae are enclosed in parentheses in the descriptions given below. All other measurements are from cercariae fixed in hot 3 percent formalin. Drawings of both living and fixed cercariae are free hand and to scale.

Identification of *A. vespertilionis* and *A. oregonense* cercariae was verified by observing their penetration and subsequent development into metacercariae in larval caddis flies. A report of these observations is given in another paper (Burns, 1961).

The form of the descriptions given here is adapted from Hall (1960) to facilitate comparisons with the majority of known virgulate cercariae of this country.

DESCRIPTIONS OF CERCARIAE

Allassogonoporus vespertilionis

(Figures 1 and 2)

Specific diagnosis: Body elongate-oval when extended, nearly spherical when contracted, length 0.142 to 0.177 (fully extended, 0.250 to 0.275; contracted, 0.110 to 0.122), width 0.055 to 0.067 (extended, 0.032 to 0.040; contracted, 0.065 to 0.09); covered with minute spines; with at least eight papillae with setae on each side. Tail length 0.075 to 0.105, width 0.015 to 0.020; attached posteroventrally; bearing neither spines nor bris-

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tles. Oral sucker 0.045 to 0.047 long, 0.032 to 0.04 wide. Virgula present, 0.03 to 0.035 long, 0.032 to 0.04 wide; consisting of two lobed sacs, each with medial folds and not fused posteriorly. Stylet 0.015 to 0.016 long; shoulder prominent dorsally, diminishing ventrally; ventral aspect of shaft longer than dorsal. Pharynx 0.007 to 0.012 long, 0.01 to 0.012 wide; esophagus weakly developed, birfurcating just anterior to acetabulum; ceca short and weakly developed. Acetebulum 0.017 to 0.022 in diameter; its anterior margin 0.072 to 0.091 from anterior end of body. Four pair of penetration glands anterolateral and posterior to acetabulum; first two pair similar in appearance, hyaline; last two pair also similar, faintly and finely granulated; ducts of anterior pair median to those of the others. Excretory bladder large, vesicular, distinctly epithelial; flame cell formula 2[(2+2+2)+(2+2+2)]. Sporocysts round, oval or irregular; 0.300 to 0.450 long, 0.190 to 0.255 wide; provided with a major protuberance embedded in host tissue; each sporocyst containing 6 to 15 cercariae.

Host: *Fluminicola virens* (Lea).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

Although capable of vigorous swimming activity, the cercaria of *A. vespertilionis* remained relatively quiet unless the water was agitated. Newly emerged cercariae swam towards the surface, then slowly descended while weakly bending and extending the body. Most cercariae were linked by long, fine threads of mucous which aided in keeping them suspended in water. Creeping movements by cercariae in the container were infrequent, although commonly seen in larvae under the cover-glass.

This cercaria is distinguished by its relatively short stylet in comparison to the size of the body, by the possession of four pair of penetration glands and by the ducts of the anterior pair of glands median to those of the others.

A. vespertilionis cercariae adhered, presumably by mucous threads to the surface of nearly any object drawn through the water. Stonefly (*Acroneuria* sp.) and caddis fly (*Dicosmoecus* sp.) larvae placed in a dish containing these cercariae became entangled in mucous and as a consequence became densely covered with the larval parasites. Cercariae in contact with the cuticle surrounded themselves with additional mucous and entered the gills and intersegmental areas of both of these insect larvae. Encysted metacercariae in naturally infected caddis flies developed into adults in the small intestine of the hamster (Knight and Pratt, 1955). Metacercariae from stonefly larvae also matured in the hamster, thus establishing a new intermediate host record for this parasite. Additional observations on the penetration and further development in the caddis fly are presented by the author in another paper (Burns 1961).

Acanthatrium oregonense

(FIGURES 3 and 4)

Specific diagnosis: Body elongate-oval when extended, nearly spherical when contracted; length 0.135 to 0.170 (extended, 0.155 to 0.205; contracted, 0.070 to 0.100), width 0.067 to 0.085 (extended, 0.025 to 0.040; contracted, 0.065 to 0.075); covered with minute spines; with at least eight papillae with setae on each side. Tail 0.070 to 0.114 long, 0.015 to 0.022 wide; attached posteroventrally; covered with minute spines; tip provided with enlarged bristles. Oral sucker 0.045 to 0.060 long, 0.037 to 0.050 wide. Virgula large and complex, 0.035 to 0.052 long, 0.037 to 0.050 wide; consisting of two elongated, convoluted, doughnut-shaped rings, united anteriorly by a bridge but not fused posteriorly. Stylet 0.017 to 0.022 long; tip bent ventrad, lateral borders of shaft irregular in outline. Pharynx slightly developed; esophagus and intestine not observed. Acetabulum 0.020 to 0.025 in diameter, its anterior margin 0.077 to 0.110 from anterior end of body. Three pair of penetration glands lateral and anterior to acetabulum with ducts in single bundle; anterior pair finely and densely granulated and with median lobes extending anteriorly when body extended; middle pair very small and hyaline; posterior pair coarsely granulated. Excretory bladder thin-walled, V-shaped; flame cell formula 2[(3+3+3)+(3+3)]. Sporocysts irregular in shape, 0.075 to 0.390 long; usually provided with an appendage and containing 2 to 14 cercariae.

Host: *Oxytrema silicula* (Gould).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

The cercaria of *A. oregonense* was distinguished from the other cercariae described here by its activity in water. This cercaria was constantly in motion, either swimming or creeping. When not engaged in these movements the cercaria was continually flexing and extending its body, apposing the acetabulum with the oral sucker. These cercariae frequently encysted under cover-glass pressure.

Distinguishing features include the relatively complicated virgula, the densely granulated anterior pair of penetration glands and the flame cell formula.

A. orgonense cercariae formed a firm spherical cyst on the cuticle of the gills of caddis fly larvae (*Dicosmoecus* sp.) and stonefly larvae (*Acroneuria* sp.) and the anal processes of unidentified chironomid larvae. The externally

encysted cercariae then penetrated and entered these larval insects. Further development into unencysted metacercariae was observed in caddis flies by Knight and Pratt (1955). These workers showed that a small percentage of metacercariae from naturally infected insect larvae developed into adult forms in the hamster. Additional observations on penetration by cercariae, development of metacercariae and encystment of metacercariae after pupation of the caddis fly are presented in another report (Burns, 1961).

There is a possibility that two very closely related species or perhaps two strains exist with respect to this cercaria. One appeared to be slightly larger, less active and more prone to creeping than the other. At times the middle pair of penetration glands, usually very difficult to distinguish, were more obvious in the larger form. These features were not constant enough to demonstrate the existence of two species. Both variants encysted on and penetrated the gills of caddis fly larvae.

Cercaria gyrinoides sp. n.
(FIGURES 7 and 8)

Specific diagnosis: Body elongate-oval when extended, nearly spherical when contracted; length 0.145 to 0.165 (extended, 0.180 to 0.200; contracted, 0.080 to 0.090), width 0.052 to 0.065 (extended, 0.025 to 0.035; contracted, 0.075 to 0.080); covered with minute spines; with at least eight papillae with setae on each side. Tail length 0.062 to 0.096, width 0.015 to 0.020; attached posteroventrally; tip provided with short bristles. Oral sucker 0.057 to 0.073 long, 0.042 to 0.055 wide. Virgula present, 0.048 to 0.065 long, 0.042 to 0.055 wide; consisting of two lobed sacs not fused posteriorly. Stylet length 0.020 to 0.025, tip bent slightly ventrad. Pharynx weakly developed; esophagus and intestine not observed. Acetabulum 0.018 to 0.025 in diameter; its anterior margin 0.082 to 0.105 from anterior end of body. Three pair of penetration glands lateral and anterior to acetabulum with ducts in single bundle; anterior pair coarsely granulated; middle pair finely granulated; posterior pair hyaline. Excretory bladder thin-walled, V-shaped; flame cell formula 2[(2+2)+(2+2)]. Sporocysts not observed.

Host: *Oxytrema silicula* (Gould).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

The vigorous swimming activity of *C. gyrinoides* was sporadic, interrupted with frequent periods of rest during which the body was slowly extended and contracted. Cercariae on the bottom frequently interrupted their creeping movements by fully extending the tail and body while wiggling, resembling minute tadpoles. Cercariae under the coverglass sometimes encysted, especially when pressure was exerted or when neutral red was added. This cercaria emerged from snails throughout the year, but was most frequently encountered in late summer and early fall.

The cercaria is distinguished from others by the shape of the virgula, by the penetration grands arranged anterior to the acetabulum and by the flame cell formula.

C. gyrinoides encysted on and penetrated the cuticle of the gills of caddis fly larvae (*Dicosmoecus* sp.) and the anal processes of unidentified chironomid larvae. In this behavior they resembled the cercariae of *A. oregonense*, although they produced an external cyst that was oval rather than spherical as in the latter species. Further development of *C. gyrinoides* in the arthropod host was not followed.

Cercaria postguttata sp. n.
(FIGURES 5 and 6)

Specific diagnosis: Body elongate-oval when extended, oval when contracted; length 0.112 to 0.127 (extended, 0.178 to 0.183; contracted, 0.092 to 0.097), width 0.041 to 0.047 (extended, 0.017 to 0.025; contracted, 0.045 to 0.050); covered with very minute spins, slightly larger near the acetabulum; with at least seven papillae with setae on each side. Tail 0.070 to 0.082 long, 0.012 to 0.013 wide; attached posteroventrally; spines and bristles absent. Oral sucker 0.037 to 0.042 long, 0.031 to 0.037 wide. Virgula present, 0.032 to 0.037 long, 0.031 to 0.037 wide; consisting of two tube-like sacs with conspicuous folds, not united posteriorly. Stylet 0.012 to 0.015 long. Pharynx present; arch-shaped structure lying anterior to acetabulum may represent intestinal anlagen. Acetabulum 0.015 to 0.020 in diameter; its anterior margin 0.056 to 0.063 from anterior end of body. Three pair of penetration glands lateral and posterior to acetabulum with ducts in single bundle; first two pair similar, finely granulated; gland-cell nuclei of middle pair seen with difficulty; posterior pair hyaline. Posterior two-thirds of body filled with refractile globules. Excretory bladder thin-walled, vesicular; flame cell formula 2[(2+2+2)+(2+2)]. Sporocysts round, oval or irregular; ranging in size from 0.060 by 0.105 to 0.275 by 0.550; sometimes with appendage and containing up to 20 cercariae.

Host: *Oxytrema silicula* (Gould).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

Newly emerged specimens of *C. postguttata* were very active swimmers and seldom remained for long on the bottom of the container. Creeping movements became common some hours after emergence. Periods of swimming were fol-

lowed by intervals of rest at which time the cercaria slowly extended and contracted the body.

This cercaria is distinguished by the shape of the virgula, by the arrangement of penetration glands posterior to the acetabulum, by the presence of numerous refractile globules in the posterior portion of the body and by the flame cell formula.

C. postguttata did not enter caddis fly larvae. A single, newly encysted cercaria was found in the body cavity of a stonefly larva (*Acroneuria* sp.) which had been exposed to large numbers of cercariae 24 hours previously. No other arthropeds were tested for suitability as intermediate hosts for this form.

Cercaria guttata sp. n.

(FIGURES 9 and 10)

Specific diagnosis: Body elongate-oval when extended, nearly spherical when contracted; length 0.192 to 0.230 (extended, 0.270 to 0.315; contracted, 0.135 to 0.183), width 0.085 to 0.110 (extended, 0.037 to 0.046; contracted, 0.143 to 0.157); covered with small spines; with at least 10 papillae with setae on each side. Tail 0.095 to 0.147 long, 0.021 to 0.027 wide; attached posteroventrally; spines and bristles apparently absent. Oral sucker 0.052 to 0.062 long, 0.045 to 0.057 wide. Virgula large, 0.027 to 0.055 long, 0.037 to 0.050 wide; sac-shaped, united posteriorly. Stylet 0.017 to 0.022 long. Pharynx present; intestinal anlagen possibly present immediately anterior to acetabulum. Acetabulum 0.030 to 0.040 in diameter; its anterior margin 0.090 to 0.112 from anterior end of body. Three pair of penetration glands anterior, lateral and posterior to acetabulum; anterior pair finely and densely granulated; middle pair coarsely granulated; posterior pair hyaline. Body filled with refractile globules most densely arranged posteriorly. Excretory bladder large, vesicular with epithelial walls; flame cell formula not determined. Sporocysts not studied.

Host: *Oxytrema silicula* (Gould).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

C. guttata was a sluggish swimmer, remained suspended in water by its swimming activity only when newly emerged. Most specimens remained on the bottom, creeping in the manner characteristic of xiphidiocercariae. This cercaria emerged most frequently from snails collected in the fall and early spring.

The cercaria is distinguished by its relatively large size, by the large virgula united posteriorly and by the extensive distribution of refractile globules throughout the body.

I did not attempt to infect insect larvae with this cercaria. However, encysted metacercariae with bodies filled with refractile globules and with stylets identical to that of the cercaria were recovered from stonefly larvae (*Acroneuria* sp.) and unidentified mayfly naiads from Shot Pouch Creek.

Cercaria perpusilla sp. n.

(FIGURE 11)

Specific diagnosis: Body small, oval to elongate-oval; length 0.060 to 0.080 (extended 0.075 to 0.082; contracted, 0.045 to 0.055), width 0.030 to 0.037 (extended, 0.018 to 0.023; contracted, 0.043 to 0.047); covered with minute spines; with at least five papillae with setae on each side. Tail 0.060 to 0.097 long, 0.010 to 0.011 wide; attached posteroventrally; with minute spines but no bristles. Oral sucker 0.016 to 0.020 in diameter. Virgula very weakly developed, represented as small lobes on lateral and posterior walls of oral sucker. Stylet 0.011 to 0.015 long. Pharynx present; esophagus and intestinal anlagen weakly developed. Acetabulum 0.010 to 0.012 in diameter; its anterior margin 0.035 to 0.050 from anterior end of body. Penetration glands posterior to acetabulum; number not determined, although two pair of ducts, presumably from glands, open on either side of oral opening. Excretory bladder V-shaped, thin-walled; main excretory ducts visible on either side of acetabulum, but minor ducts very difficult to see. Flame cell formula not determined. Sporocysts not studied.

Host: *Oxytrema silicula* (Gould).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

C. perpusilla was found infrequently. It swam vigorously and remained suspended in water. Cercariae under light cover-glass pressure survived for about 15 to 20 minutes. Cercariae frequently attached to the cover-glass by secreting material from points near the openings of the penetration gland ducts. The cercaria is distinguished by its relatively small size and by its poorly developed virgula.

Mixed infections in snail hosts

Because of their superficial resemblances to one another the various virgulate cercariae that emerged from *Fluminicola virens* and *Oxytrema silicula* were somewhat difficult to segregate. This difficulty was compounded in that a snail frequently shed two species of virgulate cercariae and infrequently three. Although the incidence of mixed infections was not accurately determined, it was sufficiently frequent to make the isolation of singly infected snails quite difficult.

DISCUSSION

The descriptions of *Allassogonoporus ves-*

pertilionis and *Acanthatrium oregonense* given here differ considerably from the original descriptions by Knight and Pratt (1955). The virgula organ, the most valuable single structure for identifying these cercariae, was inadequately described by them. They reported three pair of penetration glands for *A. vespertilionis* and two pair for *A. oregonense*. An additional pair of glands occurs in each cercaria. Knight and Pratt were unable to find traces of the V-shaped excretory bladder in *A. oregonense* and apparently did not observe flame cells in either form. One reason for the difference in the two descriptions resides in the manner in which the cercariae were prepared for study. Knight and Pratt used "semi-relaxed" cercariae, intravitally stained with neutral red. This dye is beneficial when differentially staining various glandular structures present in virgulate cercariae. It has the disadvantage, however, of causing distortion of some organs, particularly the virgula. The middle pair of penetration glands of *A. oregonense*, very difficult to see in newly emerged, unstained cercariae, is even more obscure in stained specimens. The need to supplement descriptions of living cercariae with fixed specimens to establish some standard for comparison, particularly regards to measurements, was stressed by Hall (1959).

The morphological and behavioral similarities shared by *A. oregonense* cercariae and *Cercaria gyrinoides* suggest that they may be closely related species. Both encyst on the cuticle of caddis fly larvae and enter that host. If *C. gyrinoides*, like *A. oregonense*, remains as an unencysted metacercaria in larval caddis flies, both forms may be present in naturally infected hosts. The depletion of the contents of the virgula and the penetration glands in such metacercariae would result in their being very similar in appearance. A comparative study of the arrangement and number of atrial spines in adult specimens of *Acanthatrium* recovered from hamsters fed naturally infected caddis fly larvae has suggested that another species in addition to *A. oregonense* may be present (unpublished data). An investigation of the fate of *C. gyrinoids* in uninfected caddis fly larvae should be conducted in view of these possibilities.

As indicated earlier, three different virgulate cercariae commonly emerged from the snail *Fluminicola virens* from this area. One is the cercaria of *Allassogonoporus vespertilionis*, redescribed here. Another is the cercaria of *Cephalophallus obscurus*, described by Macy and Moore (1954). The third form has not been described. It has a clover-leaf-shaped virgula which readily distinguishes it from the other two.

Hall (1959), in his discussion of taxonomic relationships in the family Lecithodendriidae, offered some preliminary suggestions regarding relationships based on known life histories. Certain of these must now be modified in light of the redescriptions of the cercariae of *A. vespertilionis* and *A. oregonense* presented here. Hall pointed out that the members of the subfamily Pleurogenetinae Looss with known life histories developed from cercariae with 4 pair of penetration glands. As one of two exceptions to this he listed *A. vespertilionis*, based on the description of the cercaria by Knight and Pratt (1955). As shown in the present report the cercaria of this trematode has 4 pair of penetration glands and is not, therefore, an exception. Hall also indicated that the three virgulate cercariae for which both the life history and flame cell pattern were known have a formula of $2[(2+2+2)+(2+2+2)]$. To these may now be added *A. vespertilionis*. The cercaria of *A. oregonense*, however, with a flame cell pattern of $2[(3+3+3)+(3+3)]$, departs from this scheme as well as from other known lecithodendriid patterns. The flame cell formulae for *Cercaria gyrinoides* and *C. postguttata* offer still different variations, although these forms may not be cercariae from this group of trematodes. These observations help support Hall's conclusion that flame cell patterns are not necessarily correlated with the morphological characters which divide the lecithodendriids into subfamilies.

Hall (1960) included *A. vespertilionis* and *A. oregonense* in a key to the virgulate xiphidiocercariae of North America. The redescriptions of these two cercariae given here invalidate their positions in the key.

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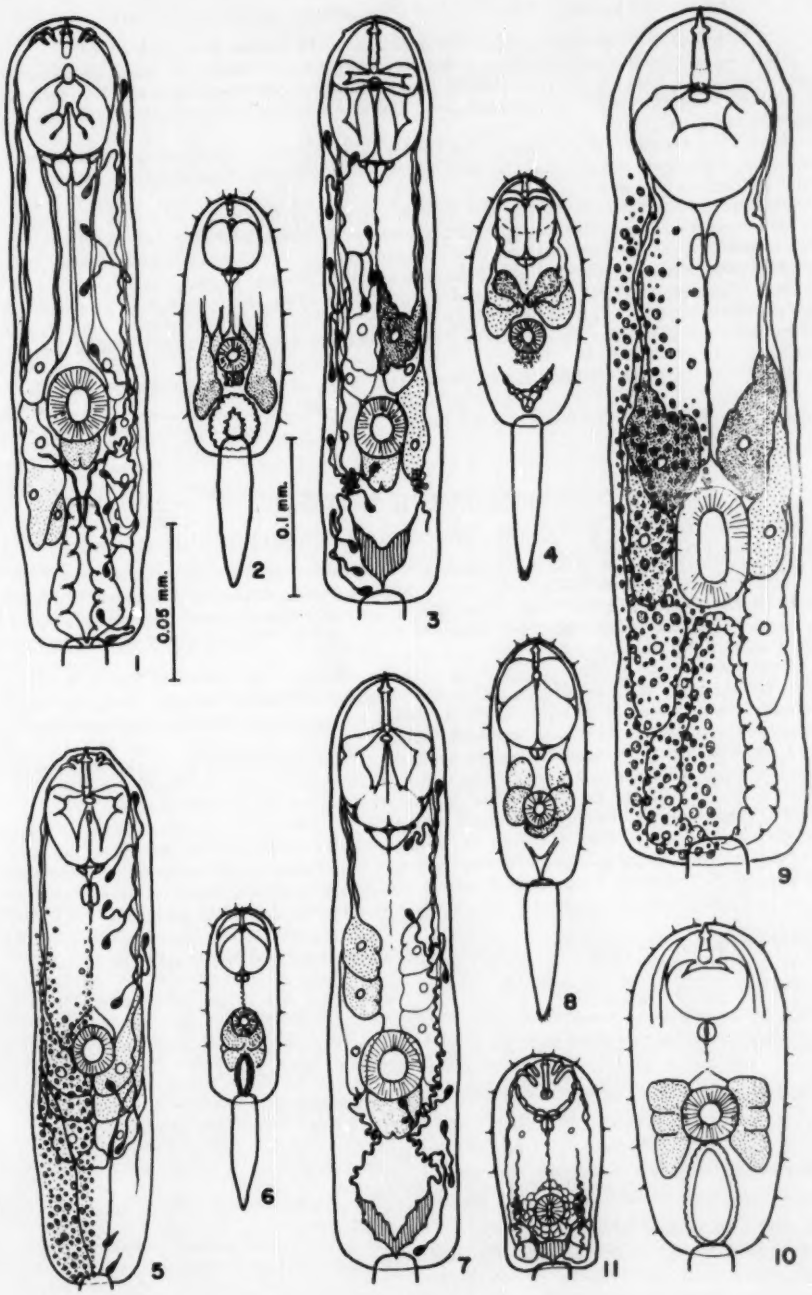
EXPLANATION OF PLATE I

FIGURES 1, 3, 5, 7, 9, and 11 from living specimens to scale shown by figure 1.
FIGURES 2, 4, 6, 8, and 10 from formalin-fixed specimens to scale shown by figure 2.
FIGURES 1 and 2. *Allassogonoporus vespertilionis.*
FIGURES 3 and 4. *Acanthatrium oregonense.*
FIGURES 5 and 6. *Cercaria postguttata.*
FIGURES 7 and 8. *Cercaria gyrinoides.*
FIGURES 9 and 10. *Cercaria guttata.*
FIGURE 11. *Cercaria perpusilla.*

PLATE I



ANNOUNCEMENT

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RESEARCH NOTE

A MALARIA SURVEY IN LIBERIA.

Liberia on the west coast of Africa between 4°22′ and 8°50′ north latitude and between 7°30′ and 11°32′ west longitude has 43,000 square miles and is bounded by the Atlantic ocean, Sierra Leone, Guinea, and the Ivory coast. The climate is equatorial with an annual rainfall of 180 inches on the coast. The rainy season is from June to September and the dry season from December to March.

During the month of January 1961 the authors under the supervision of Dr. R. S. Bray of the Liberian Institute of Tropical Medicine surveyed the native villages along the coast of Bassa County, about 70 miles south of Monrovia the capital of Liberia. Only 3 of the 17 villages surveyed were sprayed in 1956 with DDT.

A total of 500 thick malarial smears was taken from the population in the villages including infants, children and adults. The slides were stained with Giemsa's stain and examined within 2 weeks of the survey. The results are recorded in the table below.

It is interesting to compare the results with those of Young and Thomas (1949, J. Natl. Mal. Soc. 8: 247–266). They surveyed the entire country taking 10,128 blood smears and found a total incidence of 30.6 percent. The species were as follows: 83.8 percent *P. falciparum*, 14.2 percent *P. malariae*, 2.0 percent *P. vivax*, and one case of *P. ovale*.

It is obvious from these surveys that malaria is still very prevalent in parts of Liberia.

TABLE I. *MALARIAL INCIDENCE IN VILLAGES OF BASSA COUNTY, LIBERIA (1961).*

Age	Examined	Malaria positive	P. falc.	P. mal.	P. ovale	P. falc. P. mal.	P. falc. P. ovale	P. mal. P. falc. P. ovale
Under 1 yr	24	8	8	1	—	1	—	—
1–10 yrs	139	95	86	15	2	6	2	1
11–29 yrs	164	39	37	2	—	—	—	—
30 yrs and over	173	34	32	3	1	2	—	—
Total no.	500	176	163	21	3	9	2	1
Total pct.		35%	92.5%	11.9%	1.7%	5.1%	1.1%	0.6%
Young and Thomas (1949)		30.6%	83.8%	14.2%				

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PENETRATION AND DEVELOPMENT OF *ALLASSOGONOPORUS VESPERTILIONIS* AND *ACANTHATRIUM OREGONENSE* (TREMATODA: LECITHODENDRIIDAE) CERCARIAE IN CADDIS FLY LARVAE

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Knight and Pratt (1955) described the life histories of *Allassogonoporus vespertilionis* Macy and *Acanthatrium oregonense* Macy, two lecithodendriid trematodes of bats. They reported that sporocysts of both flukes produced virgulate xiphidio-cercariae that developed into metacercariae in caddis fly larve. The metacercariae of *A. vespertilionis* encysted in the insect host whereas those of *A. oregonense* existed as unencysted, motile larvae. Adults of both species developed in hamsters which were fed metacercariae from naturally infected *Limnophilus* sp. larvae.

While re-examining the life histories of these trematodes I have observed certain aspects of their development which were not reported by Knight and Pratt. These observations are presented in this report. They include the penetration of caddis fly larvae by the cercariae of both species and the encystment of *Acanthatrium oregonense* metacercariae during pupation of the insect host. An emendation of the original description of the motile metacercariae of *A. oregonense* is also given here.

MATERIALS AND METHODS

The snail hosts for *Allassogonoporus vespertilionis* and *Acanthatrium oregonense* are *Fluminicola virens* (Lea) and *Oxytrema silicula* (Gould), respectively. Infected snails were collected from Shot Pouch Creek, near Burnt Woods, Lincoln County, Oregon. Uninfected caddis fly larvae (*Dicosmoecus* sp.) were collected in mid July 1960 from Horse Creek, near its junction with the McKenzie River at McKenzie Bridge, Lane County, Oregon. Snails and caddis flies were kept in separate aerated aquaria at approximately 60 C.

Cercariae emerged from infected snails isolated in dishes kept at room temperature overnight. Penetration of the gills and abdominal wall by cercariae was observed in living caddis fly larvae removed from their stone cases and placed in dishes of stream water containing the larval parasites. For more detailed observations of the penetration process, extirpated caddis fly gills were placed on a slide in a drop of water containing numerous cercariae and covered with a cover-glass. Metacercariae were studied alive in amphibian Ringer's solution and after fixation in hot 3 percent formalin.

All measurements were taken with an ocular micrometer and are reported in millimeters. Drawings are free hand and to scale.

OBSERVATIONS

Penetration by cercariae

The cercaria of *Acanthatrium oregonense* was distinguished from other xiphidiocercariae shed by *O. silicula* and from the cercaria of *Allassogonoporus vespertilionis* by its habit of constantly bending its body to appose the oral sucker and acetabulum. Cercariae of both species secreted mucous that formed a complex of delicate threads to which the larvae adhered. These threads of mucous appeared to enhance their chance of sticking to the gills of caddis fly larvae. Kruidenier (1951) observed similar secretions from virgulate cercariae and suggested that the threads were produce by the virgula.

On contacting the gills of a caddis fly larva, *A. vespertilionis* cercariae secreted large amounts of additional mucous (fig. 1). As cercariae became surrounded in mucous their tails were detached. Frequently, many cercariae formed aggregates, adhering to one another by their mucous capsules. Encapsulated cercariae eventually became oriented with their stylets directed toward the host tissue. By alternately pushing and scraping the stylet against the host cuticle, a cercaria made an opening in the gill large enough to pass through. The process of penetration was usually completed in from 5 to 10 minutes. If penetration was not effected in that time the cercaria became moribund. Where aggregates of cercariae were formed only those larvae lying against the host succeeding in penetrating. Some members of the aggregate frequently penetrated and entered the mucous

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cyst formed by another. Cercariae located a few millimeters from the gill did not produce mucous cysts or shed their tails.

Cercariae of this species also penetrated the gills and intersegmental areas of stonefly larvae (*Acroneuria* sp.) from Shot Pouch Creek. Metacercariae from naturally infected stonefly larvae developed to adults in the small intestine of the hamster.

Acanthatrium oregonense cercariae, upon contacting the gills, often responded immediately. Adhering to the gill by mucous threads, the cercaria contracted with its ventral surface against the gill. A delicate cyst wall formed almost instantaneously, producing a dome-like capsule over the larval parasite. The tail of the cercaria was shed. The parasite then twisted and turned within the initial cyst and added more substance to the wall. The completed cyst was spherical and firmly attached to the gill surface (fig. 2). Encystment was sometimes completed in one minute. The act of penetration was similar to that described for *A. vespertilionis*. Encysted cercariae became moribund within 10 minutes if they were unable to penetrate the insect. Cercariae sometimes left the initial, thin-walled cyst and moved along the gill surface. These tailless forms did not attempt to re-encyst and soon became inactive. *A. oregonense* cercariae never assembled in aggregates during the encystment process. They never penetrated the host without first encysting. Cercariae also encysted on and penetrated gill remnants of molting *Dicosmoecus* sp. pupae, the gills of stonefly larvae (*Acroneuria* sp.) and the anal processes of some unidentified chironomid larvae. They sometimes encysted spontaneously under a cover-glass and on the surface of air bubbles.

Another virgulate cercaria, similar in appearance to *Acanthatrium oregonense* and which also developed in *Oxytrema silicula*, penetrated caddis fly larvae and chironomid larvae in much the same manner. It produced an oval rather than a spherical cyst. This cercaria will be described in another report (Burns, 1961).

Host reaction to penetration

A local host reaction to the invasion of *A. oregonense* cercariae was frequently observed in the caddis fly. The hypodermis adjacent to the penetrating cercaria became thickened and reddish-brown in color (fig. 2). This reaction was often rapid, in which case the cercaria failed to penetrate. The abdomen and gills of caddis fly larvae exposed to cercariae 24 hours previously were densely sprinkled with these pigmented spots. Most of the external cysts produced by the cercariae were gone by this time. Cercariae which had entered the gill were sometimes encased and immobilized in a thick layer of reddish-brown material presumed to be of host origin. Unidentified chironomid larvae, exposed to *A. oregonense* cercariae, reacted in much the same manner but to a greater degree. Few cercariae were able to enter this host; most that did enter were encapsulated through the host reaction described. The fate of this cercaria in the stonefly was not investigated.

Development of metacercariae

The encysted metacercaria of *Allassogonoporus vespertilionis* was described by Knight and Pratt (1955) from naturally infected *Limnophilus* sp. larvae. They reported the diameter of the mature cyst as 0.142 mm, but did not describe newly encysted forms. I found encysted metacercariae in the body cavity of *Dicosmoecus* sp. larvae 4 hours after exposure to cercariae. The virgula was still recognizable and the stylet was in place. The cysts measured 0.110 to 0.117 in broadest diameter. Cysts were slightly larger 24 hours after exposure, the contents of the virgula were depleted and the stylet was often lying free in the cystic fluid. Twenty-one and 42 days after exposure the size range was 0.125 to 0.135.

The following experiment determined the approximate length of time in the intermediate host required for *A. vespertilionis* metacercariae to become infective. Fifteen *Dicosmoecus* sp. larvae were exposed for 6 hours to snails discharging cercariae. Fifteen more larvae were kept free from cercariae and used as controls. Twenty-one days later, 10 caddis flies from each group were examined for metacercariae. In the exposed group, one larva had no cysts, three larvae and six pupae had few to many cysts. No cysts were found in two larvae and eight pupae from the control group. One larva and four pupae from each group were fed to hamsters. Over 200 sexually mature *A. vespertilionis* were recovered from the small intestine of the hamster fed caddis flies exposed to cercariae. No trematodes were found in the hamster fed insects from the control group.

As reported by Knight and Pratt (1955), *Acanthatrium oregonense* cercariae do not

encyst after entering caddis fly larvae. They described the motile metacercariae from a naturally infected caddis fly dissected in water. I found that motile metacercariae become distorted after a short time in water. I submit, therefore, a redescription from living specimens in amphibian Ringer's and from specimens fixed in hot 3 percent formalin. The metacercariae described were from caddis fly larvae exposed to cercariae 31 days previously. The measurements in parentheses refer to living forms, all others are from formalin fixed material.

Body elongate-oval, length 0.252 to 0.307 (extended, 0.425; contracted, 0.150), width 0.105 to 0.137 (extended, 0.080; contracted, 0.130); cuticle smooth. Oral sucker 0.042 to 0.047 in diameter; without virgula or stylet. Pre-pharynx apparently absent. Pharynx 0.015 to 0.025 long; esophagus 0.027 to 0.04 long; ceca 0.042 to 0.05 long, sometimes bulbous. Acetabulum 0.032 to 0.037 in diameter, its anterior margin 0.117 to 0.115 from anterior end of body. Excretory bladder V-shaped, distinctly epithelial, arms extending anteriorly to lateral margins of acetabulum. Primordial testes located just anterior to arms of bladder. Presumed genital atrium primordium anterior to acetabulum. Illustrated in figure 3.

On 23 August, 1960, 10 caddis fly larvae were exposed to *Acanthatrium oregonense* cercariae for 12 hours. Thirty-one days later, 5 insects were still in the larval stage, 3 had developed into pupae and 2 into imagos. The larvae and pupae harbored motile metacercariae described above. The imagos contained only encysted metacercariae (Fig. 4). The cysts were located in the thoracic musculature. The metacercariae were enveloped in a thin, firm cyst wall which was itself surrounded by a thick, gelatin-like coating. The inner cyst was spherical to oval, 0.115 to 0.210 long by 0.102 to 0.175 wide. The outer cyst was more irregular in shape, 0.150 to 0.375 long by 0.137 to 0.225 wide. The oral sucker was broader than long, 0.037 to 0.077 by 0.025 to 0.042. The diameter of the acetabulum was 0.033 to 0.038. The conspicuous V-shaped excretory bladder was filled with refractile globules.

Ten cysts were fed to a hamster. One week later, seven adult *A. oregonense* were recovered from the small intestine. Knight and Pratt (1955) showed that a small percentage of motile metacercariae of *A. oregonense* developed to sexual maturity in the hamster. These authors did not, however, observe the encysted form in caddis fly imagos.

One other caddis fly larva was abundant in the stream from which *Dicosmoecus* sp. were obtained. The latter inhabited firm, stone cases attached to the undersides of rocks. The other species was motile and built a fragile case made of stones and fir needles. Specimens of these did not survive laboratory conditions and were not identified. More than 20 of them were examined soon after they were collected and each harbored 10 or more unencysted *A. oregonense* metacercariae. None of the *Dicosmoecus* sp. larva, except those subsequently exposed to cercariae experimentally, were infected. Attempts to infect *Dicosmoecus* sp. with *A. oregonense* cercariae in late July were unsuccessful. Encystment on the gills readily occurred and penetration frequently followed. Examination 24 hours after exposure revealed that the cercariae which had penetrated were either dead or walled off and immobilized through the host reaction described above. Yet, 1 month later, specimens from the same group were susceptible to infection. *Dicosmoecus* sp. imagos were collected from the same location in late September and found infected with *A. oregonense* cysts. These initial observations suggest that these larvae, when young, were somehow resistant to infection by *A. oregonense* but were later susceptible.

DISCUSSION

Several investigators have observed xiphidiocercariae penetrating the integument of various second intermediate hosts. I have found no description of cercariae encysting on their host before penetrating. Knight and Pratt (1955) reported that the cercariae here discussed contacted caddis fly larvae but they did not witness penetration. *Oxytrema silicula* releases four different species of virgulate cercariae in addition to *Acanthatrium oregonense*. *Fluminicola virens* releases at least two virgulate cercariae in addition to *Allassogonoporus vespertilionis*. A single snail of either species was often infected with two and exceptionally three different larval trematodes, each producing virgulate cercariae. Hence, it is possible that Knight and Pratt may have been dealing with cercariae other than *A. oregonense* and *A. vespertilionis* when they at-

tempted to observe penetration. Possible mistaken identity is further indicated from my observations on the morphology of the cercariae of these two trematodes which differ from those given by Knight and Pratt. Emendations of their descriptions will be reported elsewhere (Burns, 1961).

The source of the material comprising the external cyst is not known, although the short time required for its formation suggests that cystogenous glands were involved. The virgula appeared unchanged in cercariae that had recently penetrated, indicating that that organ contributed little or nothing to either the encystment or penetration process. This observation is in accord with those of Seitner (1945) and Kruidenier (1951). The cysts formed under cover-glass pressure by *A. oregonense* were similar to those formed on the host. Kruidenier (1951) and Hall (1960a) described virgulate cercariae which also encysted spontaneously under cover-glass pressure. It will be of interest to know if the cercariae studied by Hall and Kruidenier encyst on the host before penetrating.

Two aspects of the penetration process may serve to restrict *Acanthatrium oregonense* cercariae to particular hosts; encystment on a suitable host cuticle and the defense reaction of the host. At present, conclusions regarding the roles played by each in determining host-specificity would be premature. The reaction of *Dicosmoecus* sp. to penetrating cercariae may be a wound-healing mechanism or a response to enzymatic secretions presumably injected by cercariae as an aid to penetration. Texts on insect pathology gave no description of a response corresponding to that given here. Wound-healing processes approach this response but are considerably slower.

An unencysted metacercarial stage in the development of lecithodendriid trematodes appears not to be uncommon. Hall (1960b) described two motile lecithodendriid metacercariae and showed that *Eumegacetes* sp. metacercariae may be encysted or not. Okabe (1937) reported that *Loxogenes liberum* cercariae did not encyst in dragonfly naiads until 15 to 17 days after entering that host. Encystment of *Prosthodendrium anaplocami* metacercariae occurred only when mayfly nymphs had undergone subimaginal ecdysis (Etges, 1960). Brown (1933) observed that *Lecithodendrium chilostomum* cercariae entered caddis fly larvae, overwintered as motile metacercariae in the larval insect and finally encysted in the thoracic muscles of the imagos. The development of *A. oregonense* metacercariae, described here, resembles that of *L. chilostomum*, although the caddis fly *Dicosmoecus* sp. completes its metamorphosis in a single season.

SUMMARY

The process of penetration of caddis fly larvae by the cercariae of *Allassogonoporus vespertilionis* and *Acanthatrium oregonense* is described. The former cercaria becomes encapsulated in its mucous secretions before entering the intermediate host wherein it soon encysts. The latter cercaria produces a firm, spherical cyst on the cuticle of larval caddis flies. It then penetrates the insect larva and develops into a motile, unencysted metacercaria. This larval form is redescribed. The motile metacercaria encysts in the thoracic muscles of the caddis fly imago.

ACKNOWLEDGMENT

This study was conducted while a National Science Foundation Postdoctoral Fellow in Zoology. The author is indebted to Prof. Ivan Pratt for providing aid, laboratory facilities and support from his grant (E 867) from the National Institutes of Allergy and Infectious Diseases, U. S. Public Health Service.

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EXPLANATION OF PLATE I

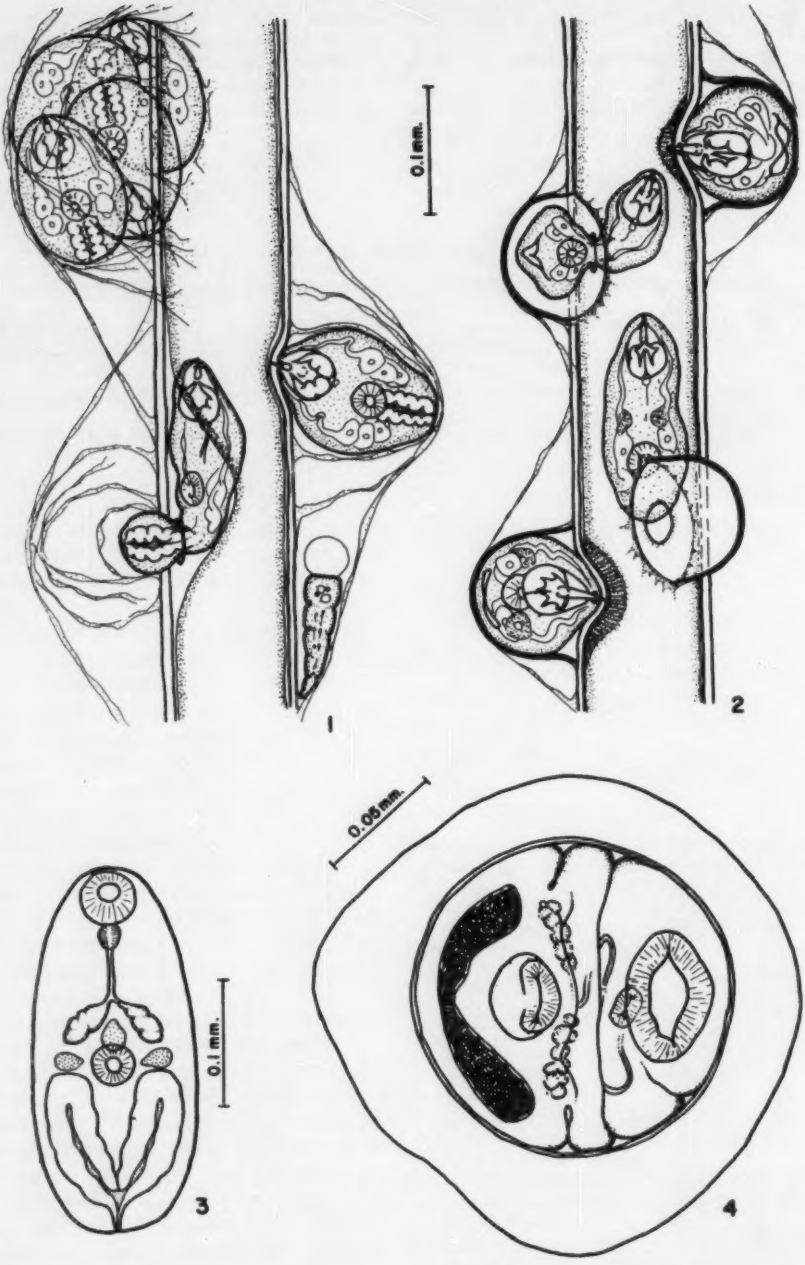
FIGURE 1. *Allassogonoporus vespertilionis* cercariae penetrating gill of *Dicosmoecus* sp. larva.

FIGURE 2. *Acanthatrium oregonense* cercariae encysting on and penetrating gill of *Dicosmoecus* sp. larva. Thickening of host hypodermis shown near cercariae at lower left and upper right.

FIGURE 3. Unencysted metacercaria of *Acanthatrium oregonense* from *Dicosmoecus* sp. larva. Formalin-fixed.

FIGURE 4. Encysted metacercaria of *Acanthatrium oregonense* from *Dicosmoecus* sp. imago.

PLATE I



THE LIFE HISTORY OF *SPHAERIDIOTREMA SPINOACETABULUM* SP. N. (TREMATODA: PSILOSTOMIDAE) FROM THE CECA OF DUCKS

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Rudolphi described *Distomum globulum* from the small intestine of *Anas fuligula* in 1819. Braun (1902) and Odhner (1913) redescribed the trematode from specimens previously collected and preserved by Creplin. Odhner established the genus *Sphaeridiotrema* with Creplin's specimens, although both Odhner and Braun lamented the poor condition of the material available for study; their redescriptions were necessarily incomplete. Price (1934) redescribed the trematode from specimens collected in Maryland. His account is by far the most complete and represents the only description with which new specimens can adequately be compared for identification.

Szidat described the life history of *Sphaeridiotrema globulum* in 1937. He determined that the cercaria involved was *Cercaria helvetica* XVII described by Dubois (1929). Szidat observed that the cercaria, after emerging from the snail *Bithynia tentaculata,* entered the mollusc and encysted between the shell and the mantle. Encysted cercariae developed into adults in the lower small intestine of domestic ducks.

Snails from the area of Corvallis, Oregon, harbor encysted metacercariae similar to those described by Szidat (1937). The metacercariae, when fed to parasite-free domestic ducks, matured in the ceca. The adult trematodes differed only slightly from *S. globulum* as redescribed by Price (1934). A study of the larval stages of the parasite from this area revealed that the cercaria was quite different from the cercaria determined by Szidat to be involved in the life history of *S. globulum*.

On the basis of these differences, a new species of the genus *Sphaeridiotrema* is here described with a partial account of its life history.

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METHODS AND MATERIALS

The description of the adult trematode was obtained from living specimens and from specimens fixed in hot AFA and stained with Delafield's hematoxylin. All adults were from the ceca of laboratory-reared domestic ducks (*Anas boschas domestica*) fed metacercariae from naturally infected snails.

The snails involved in the life history of this fluke, *Fluminicola virens* (Lea) and *Oxytrema silicula* (Gould), were collected from Shot Pouch Creek, near Burnt Woods, Lincoln County, Oregon. Rediae and cercariae occurred only in *F. virens*, although both snails harbored metacercariae. Rediae were studied alive from snails dissected in amphibian Ringer's solution. Cercariae emerged from snails isolated in dishes containing stream water at room temperature. They were studied alive and after fixation in hot 3 percent formalin. The esophagus and ceca of the cercaria were obscured by the presence of dense cystogenous gland cells. The contents of these glands were depleted when powdered charcoal was added to water containing living cercariae. The digestive tract of the cercaria was then easily seen. Measurements of cercariae were from formalin-fixed specimens. Encysted metacercariae, being neither attached to nor imbedded in host tissue, were readily obtained for study by simply removing the shell from either of the two snails in a dish of water.

All measurements were taken with an ocular micrometer and are reported in millimeters. Drawings are free hand and to scale. Measurements of the type specimen given in parentheses.

Sphaeridiotrema spinoacetabulum sp. n.

FIGURE I

Diagnosis: Psilostomidae; Sphaeridiotrematinae. Body large, ovoid to pyriform; length 1.080 to 1.404 (1.242), width 0.583 to 0.778 (0.659); widest at posterior border of acetabulum. Cuticle without spines except surrounding opening of acetabulum. Oral sucker 0.075 to 0.140 (0.140) long, 0.100 to 0.175 (0.175) wide; terminal with subterminal opening. Prepharynx very short. Pharynx well developed, 0.072 to 0.087 (0.082) in diameter. Esophagus short, longitudinally compressed in fixed specimens. Ceca extend to anterior border of posterior testis. Acetabulum large, gen-

erally broader than long, equatorial; length 0.205 to 0.285 (0.260), width 0.235 to 0.375 (0.345). Testes spherical to slightly irregular, subequal, overlapping to tandem, median and posterior. Anterior testis 0.225 to 0.275 (0.260) long, 0.225 to 0.300 (0.300) wide; posterior testis 0.235 to 0.300 (0.300) long, 0.250 to 0.350 (0.350) wide. Genital pore sub-median, on a level with and to the left of the pharynx. Genital atrium short. Cirrus pouch club-like, extending dorsally to anterior margin of acetabulum or slightly beyond; posterior half containing seminal vesicle, anterior portion containing cirrus. Ovary dorsal, sub-median, between acetabulum and anterior testis, 0.095 to 0.150 (0.120) in diameter. Seminal receptacle small, appearing as a dilatation of the oviduct near the ovary. Laurer's canal present, entering oviduct near seminal receptacle. Oviduct receiving short, common vitelline duct just beyond entrance of Laurer's canal, then becoming modified as ootype, latter encircled by large cells comprising Mehlis' gland. Uterus with few windings and containing few (two to six) large, undeveloped eggs; distal end modified as metraterm. Vitelline follicles large, six to seven on each side, C-shaped, concavity enclosing ceca along their entire length. Excretory system with two wide, anterior, dorsolateral (anastomosing?) sinuses that narrow in acetabular region into two lateral ducts, latter uniting close to dorsal, subterminal excretory pore. Eggs operculate, 0.100 to 0.115 long, 0.060 to 0.075 wide.

Host: *Anas boschas domestica.*

Location: Ceca.

Locality: Area of Shot Pouch Creek, Lincoln County, Oregon. (Locality of larval infections in snails.)

Type: U. S. National Museum Helminthological Collection No. 39807.

Paratypes: U. S. National Museum Helminthological Collection No. 39808; and collections in Department of Zoology, Oregon State University and Department of Zoology, University of Wisconsin.

The ranges of measurements given in the above description were from 10 specimens that were compressed to show details of anatomy. The following measurement ranges were from 10 specimens that were not severely compressed and are given to supplement the description (figs. 2 and 3).

Body length 0.756 to 0.896, width 0.421 to 0.540; oral sucker length 0.100 to 0.135, width 0.115 to 0.155; acetabulum length 0.190 to 0.230, width 0.205 to 0.260; pharynx 0.075 to 0.090 in diameter; ovary 0.093 to 0.132 in diameter; anterior testis length 0.185 to 0.255, width 0.150 to 0.250; posterior testis length 0.175 to 0.235, width 0.180 to 0.270.

This species is apparently very closely related to *S. globulum* and, based on the incomplete redescriptions of that trematode by Braun (1902) and Odhner (1913), impossible to distinguish from it. The measurements and relative positions of the major organs of *S. spinoacetabulum* given here agree with those given by Odhner and by Braun. They were, however, unable to find cuticular spines on their specimens, did not describe the ceca, seminal receptacle, Laurer's canal or Mehlis' gland, and gave no measurements of the testes or ovary. *S. spinoacetabulum* is very similar to *S. globulum* as redescribed by Price (1934) except that his specimens did not possess cuticular spines around the acetabulum and apparently lacked the seminal receptacle and Laurer's canal. He also described the uterus as containing 60 eggs, although that figure very likely is a misprint. The omissions in the earlier descriptions of *S. globulum* coupled with the more evident differences between its cercaria and that described below for *S. spinoacetabulum* form the basis for establishing a new species.

LIFE HISTORY

Redia

The development of the egg and miracidium was not observed. No sporocyst was found in 15 *Fluminicola virens* infected with *S. spinoacetabulum*. Mature rediae (fig. 5), filled with cercariae, occurred throughout the visceral organs of the snail host but were most numerous in the gonad. No redia contained daughter rediae. The mature redia was sacciform and elongated with two ventral lobes near the posterior end. A birth pore opened just posterior to a collar-like band near the anterior end. The shape and size of the redia was highly variable. The length of 20 rediae containing cercariae that were near maturity ranged from 0.378 to 1.620; the width from 0.130 to 0.486. A muscular, knoblike projection at the anterior end was pierced by a short canal that led to the ovoid pharynx. The pharynx was 0.039 to 0.075 long, 0.033 to 0.065 wide. The sacciform intestine extended from the pharynx to the midregion of the body or beyond to the ventral lobes. The contents of the intestine were usually bright orange, resembling the color of the gonad of *F. virens*.

Immature rediae (fig. 4) ranged in length from 0.175 to 0.205; in width from 0.075 to 0.087. They were quite active and capable of extending the body to a length of 0.300 and contracting to 0.150. The ventral lobes and col-

lar band were more distinct than in mature rediae. The intestine extended to the level of the ventral lobes.

Cercaria (Fig. 6)

Diagnosis: Body flattened, ovoid, 0.325 to 0.375 long, 0.197 to 0.220 wide; cuticle thick, without spines but with at least 7 papillae bearing setae on each side. Tail subterminal, 0.475 to 0.605 long, 0.050 to 0.060 wide; margins clear, suggesting short lateral fins; with canal (excretory duct?), bifurcated distally, running through the center to a distance of 0.195 to 0.275 from base. Oral sucker terminal with subterminal opening; sometimes protruded from anterior end; 0.052 to 0.063 long, 0.047 to 0.060 wide. Acetabulum large, postequatorial; 0.050 to 0.075 long, 0.072 to 0.087 wide; its anterior margin 0.187 to 0.242 from anterior end of body. Prepharynx short. Pharynx 0.022 to 0.030 long, 0.017 to 0.022 wide. Esophagus bifurcating in mid-body region; ceca ending midway between acetabulum and posterior end of body. Excretory bladder thin-walled, vesicular to U-shaped; main excretory ducts extending anteriorly from bladder to pharyngeal region where they turn posteriorly. Dense, striated cystogenous gland cells, each with vesicular nucleus, present dorsolaterally from region of pharynx to posterior end of body; similar glands medial, between main excretory ducts from pharynx to acetabulum; gland cells less dense than preceding, each with vesicular nucleus, present laterally between excretory duct and lateral glands. Reproductive primordia between acetabulum and excretory bladder. At least 14 flame cells on each side. Small excretory ducts and possibly many flame cells obscured by dense nature of cystogenous glands.

Host: Fluminicola virens (Lea).

Locality: Shot Pouch Creek, Lincoln County, Oregon.

Cercariae of *S. spinoacetabulum* resemble echinostome cercariae without, of course, the collar of spines. They emerge from the anal opening of infected snails. The larvae are vigorous swimmers and progress rapidly through the water. The body of a swimming cercaria appears nearly spherical; the tail is not particularly conspicuous. Cercariae are positively phototropic and tend to be positively geotropic. Creeping movements by freshly emerged cercariae are never observed except when the larvae are in contact with the exposed tissues of the snail. Here they progress by means of the two suckers, the acetabulum being the primary attachment organ. Random creeping on the foot, head and mantle of the snail ends when cercariae reach the edge of the mantle next to the shell. They push back the mantle at that point, forcing their way between it and the shell. When the entire body of the cercaria is under the mantle, the tail is cast off. Approximately 2 hours after entering the snail, cercariae occurred between the mantle and the shell, near the columellar muscle, encased in thin-walled cysts. Metecercariae were encased in cysts with walls 0.005 thick 5 hours after they had entered the snail. Sixteen hours after the penetration of cercariae, the cysts were 0.148 to 0.165 in broadest diameter.

Metacercaria

Excysted metacercariae were not studied. Cysts recovered from sails exposed to cercariae 1 week earlier were 0.157 to 0.165 in broadest diameter. Cysts from snails with natural infections measured from 0.162 by 0.168 to 0.200 by 0.212. The cyst wall was 0.007 to 0.009 thick. Characteristically the metacercaria was so situated within the cyst that only the lateral aspects of the oral sucker, acetabulum and pharynx were apparent (fig. 7). On the few occasions that these organs could be seen from a dorsal view, the oral sucker was 0.040 to 0.053 long, 0.050 to 0.062 wide; the pharynx 0.023 to 0.028 in diameter; and the acetabulum 0.058 to 0.066 long, 0.078 to 0.088 wide. The most prominent feature of encysted metacercariae was the presence of large excretory sinuses that contrasted with the otherwise dense parenchyma of the larvae.

Adult *S. spinoacetabulum* were not present in young ducks fed cysts from snails exposed to cercariae 24 hours earlier. They were present in the ceca of ducks fed cysts from snails 16 days after exposure to cercariae.

Approximately 1000 cysts were present in a specimen of *Fluminicola virens* that harbored the rediae of *S. spinoacetabulum*. Most of the cysts adhered to one another in clumps that were loosely situated between the mantle and the shell, near the columellar muscle. Other cysts were cemented in masses by calcium deposits on the inner surface of the shell in this region. Encysted metacercariae were similarly located in *F. virens* that were not infected with the early larval stages of this trematode and in *Oxytrema silicula*, but fewer cysts were present in these snails. Rarely were any but the very smallest of snails from Shot Pouch Creek without metacercariae.

EFFECT OF HEAVY INFECTIONS IN DUCKLINGS

Three of seven 4-week-old ducklings died 8 days after being fed 250 infective metacercariae of *S. spinoacetabulum*. The four remaining

ducklings were killed and examined 9 days after infection. The ceca of all ducklings showed an intense hyperemia, evidence of severe hemorrhage into the lumen and marked ulceration of the mucosa. Ulcerated areas contained many adult worms situated close together and firmly attached to the mucosa by their acetabula. The ceca of the trematodes were bright red to amber, suggesting that these worms ingest blood from the host. The pathological picture was very similar to that described by Price (1934) for *S. globulum* infections in the small intestine of the lesser scaup, *Aythya affinis*. Fifteen ducks experimentally infected with *S. spinoacetabulum* metacercariae all harbored adults in the ceca. No trematode occurred in any other portion of the intestinal tract of these birds.

DISCUSSION

The morphological features of *Sphaeridiotrema spinoacetabulum* were compared with those of *S. globulum* earlier in the text and need not be recounted here. The location of S. *spinoacetabulum* in the ceca of ducks may be of some significance. The only records of *Sphaeridiotrema* sp. from the ceca are from ducks in Oregon. In 1930, E. M. Dickinson and J. N. Shaw each submitted trematodes to the United States National Museum Helminthological Collection. Dickinson's specimens (Cat. No. 29773) were from the ceca of a domestic duck at Corvallis, Oregon; Shaw's specimen (Cat. No. 30840) was from the cecum of a duck at Eddyville, Oregon. The worms were determined to be *Sphaeridiotrema* sp. by E. W. Price. Dikmans (1945) listed *S. globulum* from the ceca of ducks in Oregon, probably after examining the specimens submitted by Dickinson and Shaw, although no record of his identification appears in the Catalogue of the Helminthological Collection (M. A. Doss, person communication). The European host list (Yamaguti. 1958) for *S. globulum* includes several birds, chiefly Anatidae. In the U. S., the trematode has been reported from *Aythya affinis* by Price (1934) and Gower (1938) and from *Glaucionetta clangula americana* by Gower (1938). All of these records list *S. globulum* from the small or large intestine (one report of the latter). After examining specimens of *Sphaeridiotrema* sp. from Dr. Dickinson's personal collection, I am convinced that they are *S. spinoacetabulum* although the spines near the opening of the acetabulum were not always apparent. It seems entirely possible that *S. spinoacetabulum* is restricted to the cecum of the host whereas *S. globulum* does not occur there.

Dubois (1929) described the redia that contained *Cercaria helvetica* XVII, the cecaria determined by Szidat (1937) to be that of *S. globulum*. The description of the redia of *S. spinoacetabulum*, given here, is nearly identical with Dubois' description. His illustration of the redia, however, included a mature *C. helvetica* XVII that was unlike the cercaria of *S. spinoacetabulum*.

There are some common features shared by the cercaria of *S. spinoacetabulum* and the cercaria of *S. globulum* as described by Dubois (1929) and Szidat (1937). The nature of the cuticle and the distribution and texture of the cystogenous gland cells are alike in these two cercariae. The excretory bladder and major ducts are similar in the two forms, although these organs are filled with conspicuous refractile granules in *S. globlum*, a condition never encountered in *S. spinoacetabulum*. The body, tail, oral sucker, pharynx and acetabulum of *S. globulum* cercaria are much smaller than their counterparts in *S. spinoacetabulum*. Both Dubois and Szidat stressed the opacity of the tail of their cercaria, a condition that was not apparent in this cercaria. Dubois described a "rhabdocoele" intestine ending just in front of the ventral sucker for *C. helvetica* XVII. The esophagus of *S. spinoacetabulum* bifurcates in te midbody region and the ceca extend to near the posterior end of the body.

Szidat (1937) mentioned that the cercariae of *S. globulum*, after a short swimming session, entered *Bithynia tentaculata* and somehow attached to the innerside of the shell as well as between the shell and the mantle and encysted. He did not indicate how the cercaria entered the snail host or the length of time required for encystment. Encysted *S. spinoacetabulum* metacercariae are larger than those of *S. globulum* and lack Szidat's "spherical crystals" in the broad branches of the excretory system.

Szidat recovered adult *S. globulum* from the lower small intestine of domestic ducks fed metacercariae from *B. tentaculata*. *S. spinoacetabulum* adults, as mentioned earlier, were

found only in the ceca.

SUMMARY

Sphaeridiotrema spinoacetabulum sp. n. is described and compared with *S. globulum*, the only other species in the genus. The two species are very similar in appearance. The life history from the redia to the adult is described. The cercariae of *S. spinoacetabulum* differ conspicuously from those of *S. globulum* and provide the basis for establishing a new species. Cercariae that emerged from *Fluminicola virens* entered the snail between the mantle and the shell and encysted near the columellar muscle. Infective metacercariae developed to adults in the ceca of domestic ducks. Heavy infections of this trematode in young ducks were sometimes lethal. The ceca of infected ducks were hyperemic, hemorrhagic and ulcerated.

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EXPLANATION OF PLATE 1

FIGURE 1. *Sphaeridiotrema spinoacetabulum* adult. Details from living specimens, measurements from compressed specimens fixed in hot AFA.

FIGURE 2. Adult, very lightly compressed.

FIGURE 3. Adult, lateral view.

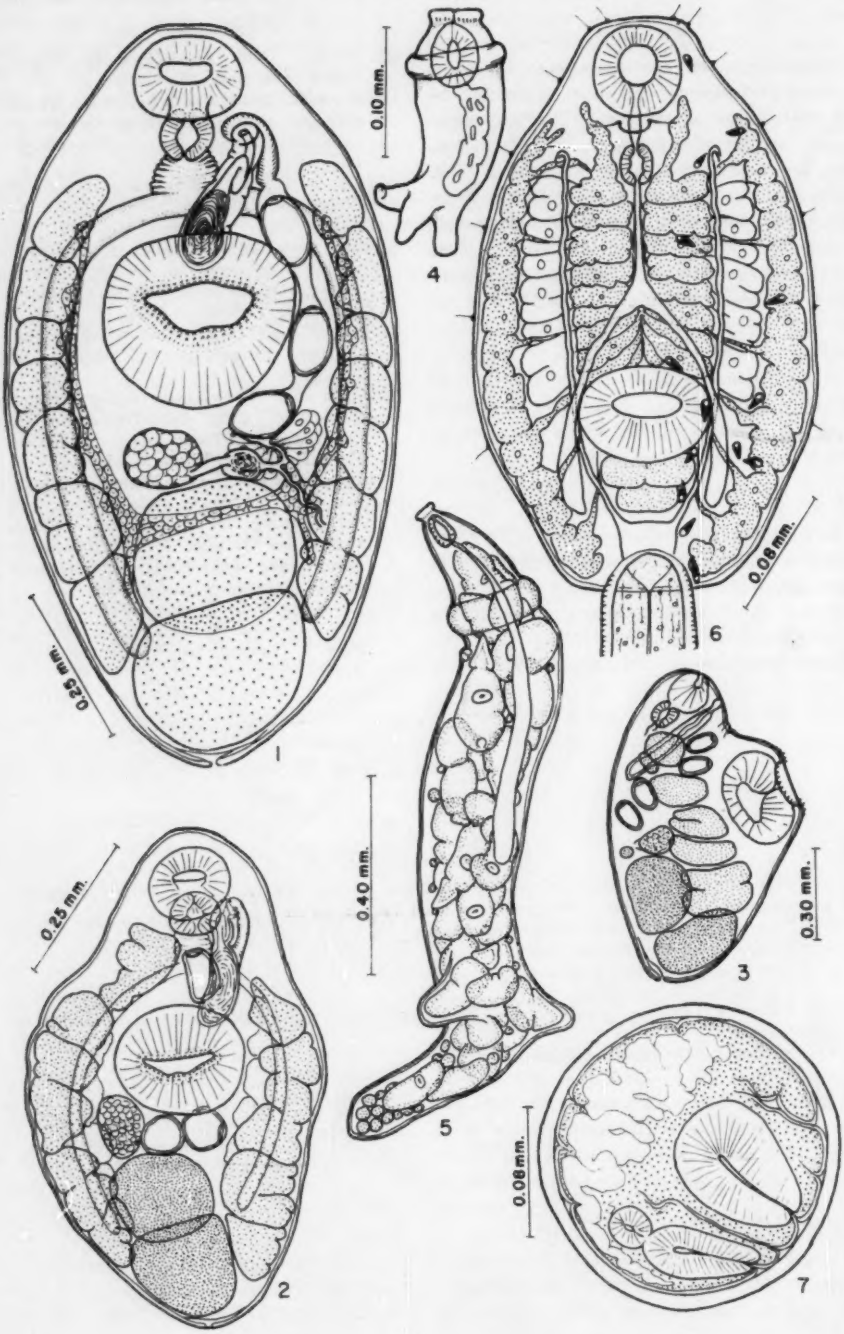
FIGURE 4. Immature redia.

FIGURE 5. Mature redia.

FIGURE 6. Cercaria. Details from living specimens, measurements from formalin-fixed specimens.

FIGURE 7. Encysted metacercaria.

PLATE I



INFLUENCES OF AGE AND SEX ON THE SUSCEPTIBILITY OF ALBINO MICE TO INFECTION WITH *ASPICULURIS TETRAPTERA**

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Albino mice demonstrate varying susceptibility to infection with the pinworm *Aspiculuris tetraptera*. Older mice are less susceptible than younger mice, and female mice are less susceptible than male mice (Mathies, 1954, 1959 a,b).

Several years ago, investigation of the mouse-*Aspiculuris* relationship was initiated in this laboratory. With the accumulation and evaluation of a growing body of data, particularly that of the controls, a pattern slowly emerged. In ths report, an attempt will be made to more fully delineate the time of onset of "age and sex resistance," and to correlate the period of infection with the degree of resistance exhibited.

METHODS AND MATERIALS

The albino mice used in all the experiments reported herein were raised in a helminth-free mouse colony maintained by the Division of Parasitology of the School of Public Health and Administrative Medicine, Columbia University. The various experiments, consisting of such procedures as prior infections with *Aspiculuris* and other helminths, splenectomy, and altered thyroid activity, were performed to observe their effects on the susceptibility of mice to a challenge infection with *Aspiculuris* (Stahl, unpublished data).

For example, a typical reinfection experiment consisted of a group of experimental mice and three groups of control mice. The first two groups of control mice were infected at the same time, with the first worm burden. However, these two groups of controls were sacrificed after different intervals of time, the one group after 2 weeks, the other group after 3, 5 or 6 weeks, depending on the length of the given experiment. In effect, the first group served as controls for the second group. The third group of mice served as controls for the reinfecting worm burden. It was from these many groups of control mice that the data on age and sex were compiled and which are presented in tables I and II.

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Aspiculuris eggs were obtained by macerating gravid female worms and then straining this material through a fine mesh brass screen. This permitted the accumulation of the eggs with very little accompanying debris. The eggs were then incubated for 7 days in distilled water at room temperature (25 C). At this point, the eggs are infective to mice (Philpot, 1924; Wells 1952). The infective eggs were gathered in centrifuge tubes and refrigerated at 4 C (Chan, 1953). Several hours prior to infecting mice, the eggs were suspended in their distilled water medium by the addition of a small quantity of gum tragacanth. Following their suspension, the eggs were warmed in a 37 C incubator for about 4 hours, then administered to the mice by intubation directly into the stomach, using a blunt, polished 18 gauge needle and a 1 cc tuberculin syringe. All mice received a standard dose of 200 infective eggs. This inoculum was equal at all times for all the experiments.

TABLE I. *Influence of host age and sex on an* Aspiculuris *infection of two weeks duration. (Standard inoculum of 200 infective eggs was given to all mice.)*

Age of mice when infected	Number and sex of mice	Mean worm burdens
3 weeks	20 male	99
	20 female	95
4 weeks	60 male	83
	60 female	79
5 weeks	30 male	60
	30 female	54
6 weeks	30 male	68
	30 female	67
7 weeks	9 male	63
	9 female	73
8 weeks	20 male	54
	20 female	53
9 weeks	10 male	48
	10 female	44
10 weeks	7 male	46
	9 female	30
15 weeks	10 male	43
	10 female	19
18 weeks	20 male	45
	20 female	18

The examination of the infected mice proceeded in the following manner. After sacrificing a mouse, by stretching its neck, the colon was removed and slit open in zinc sulphate solution, specific gravity 1.18. The worms floated to the surface of the zinc sulphate solution where they were picked up with a wooden sliver and removed to another dish. All of the examination and counting was done

under the low power of a dissecting microscope (magnification 12 ×).

RESULTS AND DISCUSSION

In the following presentation of data, mice of different sex were infected from the same inoculence and are of course, comparable. Mice of different ages are also considered comparable because it has been shown repeatedly that mice of different ages, when infected from either the same or different inoculences, will harbor different worm burdens, which are rather constant for each age group.

The figures listed in table I were obtained from mice sacrificed 2-weeks after infection. These series of animals, infected at ages ranging from 3 to 18 weeks, demonstrate that it is not until the mice are 10 weeks of age that the sexes begin to diverge in terms of pinworm burdens. These numerical data are graphically depicted in figure 1, where the age and sex

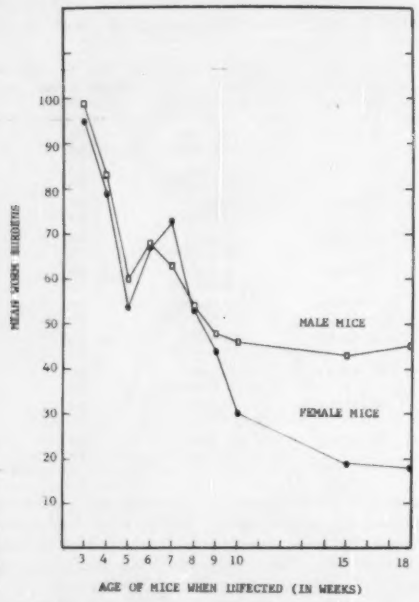

FIGURE 1. Influence of host age and sex on an *Aspiculuris* infection of 2-weeks' duration.

variations in susceptibility readily become apparent. Mice infected at 3 weeks of age reveal the greatest susceptibility to *Aspiculuris*. There is then a sharp drop in the curve for the 4- and 5-weeks-old mice of both sexes, apparently correlated with the onset of sexual maturity. With this strain of mice, the first estrus of the females usually occurs at 30 days of age, while adolescence in the males begins at about 35 days of age, with the appearance of detectable sex hormone. Mice in the succeeding age groups show an equal and constant decline in their worm burdens (age resistance) until the 10th week. At this time, a distinct difference in the susceptibility of male and female mice appears (sex resistance), and remains in evidence through the 18th week. With the appearance of sex resistance, a plateauing out of the mean worm burdens occurs.

The mean worm burdens, ranging from a high of 99 worms per mouse in the 3-week age group to a low of 18 worms per mouse in the 18-week age group, resulted from the administration of a standard inoculum of 200 infective eggs to all mice. Why the maximum return from these 200 eggs was only 50 percent is not known, although this percentage return compares favorably with that obtained by other workers (Wells, 1952; Chan, 1953, 1955; Mathies, 1954, 1959 a,b).

Mathies (1959 a,b) presented his observations on age and sex resistance to *Aspiculuris*. He infected a series of groups of mice ranging in age from 3 to 24 weeks. Almost every group showed a marked difference in the worm burdens of the male and female mice. It is important to note here that Mathies routinely sacrificed his animals after a 3-week period of infection. The distinct effects of an extra week (or weeks) of infection on a given burden are even more clearly revealed in table II. Here, two groups of mice were infected on the same day, but were sacrificed after different intervals of time. In all cases, the first of the two groups of mice was sacrificed after 2 weeks, and the

TABLE II. *Comparison of the mean worm burdens in* Aspiculuris *infections of 2-, 3-, 5-, and 6-weeks duration in albino mice. (All mice were four weeks of age when infected.)*

Numbers of mice		Period of infection	Mean worm burdens	
Male	Female		Male mice	Female mice
28	28	2 weeks	77	81
27	28	3 weeks	67	39
20	20	2 weeks	78	75
20	20	5 weeks	58	40
20	20	2 weeks	82	82
20	20	6 weeks	65	40

second group was sacrificed after 3, 5, or 6 weeks. All the mice were 4 weeks old when infected.

When the period of infection was 2 weeks long, there were no differences in the susceptibility of male and female mice. After 3 weeks, however, it can be seen that the female mice have lost 50 percent of their worms, while the male mice have lost approximately 15 percent of their worm burdens. Lengthening the interval of infection to 5 and 6 weeks did not result in any further loss of worms.

Mathies (1959 b) suggested that the female sex hormones are deleterious to *Aspiculuris*, and that these hormones are the basis of the sex resistance of female mice. It now has been shown that female mice up to the age of 10 weeks are as susceptible to an *Aspiculuris* worm burden as are the male mice, if the period of infection is 2 weeks. If the period of infection is lengthened to 3 weeks or more, then sex differences in susceptibility are noted. Mathies' hypothesis can be extended to explain these observations. The effects of the estrogenic hormones may be cumulative in nature, in that exposure to the estrogens over a period of time apparently is necessary for an expression of sex resistance. The shorter the period of infection, the higher the estrogen titers would have to be, as is probably the case with female mice ten weeks of age and older. The younger female mice, under 10 weeks of age, are not as endocrinologically mature as the older mice and require the longer period of infection to successfully affect reductions in their worm burdens.

SUMMARY

1. Albino mice, up to the 10th week of age, do not demonstrate sex differences in susceptibility to *Aspiculuris* after a 2-week period of infection.

2. If the period of infection is extended to 3 or more weeks, sex differences in susceptibility become evident, the female mice revealing worm burdens approximately one-half those of the male mice.

3. Female mice, 10 weeks of age and older, quickly evince a sex resistance to *Aspiculuris*, distinct differences in the worm burdens of male and female mice being noted after only 2 weeks of infection.

4. The degree of sex resistance to *Aspiculuris* is equated with the product of two factors, the endocrinological status of the host when infected, and the period of infection.

5. Albino mice, up to the 10th week of age, exhibit age-related decreasing susceptibility to infection with *Aspiculuris*. After the 10th week, an apparent equilibrium is reached, no further variations in age susceptibility occurring.

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RESEARCH NOTE

A TECHNIQUE OF COUNTING THE FLAGELLATE, *CRYPTOBIA SALMOSITICA*, IN THE BLOOD OF COHO SALMON.

Preliminary attempts to count the number of hemoflagellates, *Cryptobia salmositica* Katz, (1951, J. Parasit. **37**: 245–250) in the blood of infected adult coho salmon, *Oncorhynchus kisutch* (Walbaum), using samples of blood diluted with the usual blood-diluting fluids were unsuccessful. Even when wet preparations or stained smears indicated large numbers of *Cryptobia* in a blood sample, the hemoflagellates could not be distinguished in the hemocytometer. The following technique permitted an estimate of the number of *Cryptobia* per cubic millimeter of peripheral blood in infected fish.

The blood of living, sexually mature salmon was obtained by severing the caudal peduncle of the fish near the adipose fin. About 10 ml of the spurting blood was collected in sample bottles, which contained .012 g of crystalline ammonium oxalate and .008 g of crystalline potassium oxalate. These had been prepared by dissolving 1.2 g of ammonium oxalate and 0.8 g of potassium oxalate in 100 ml of distilled water. One milliliter of the solution was then placed in each sample bottle and evaporated to dryness. The oxalated blood was stored in a refrigerator at 3 C until examined.

To make the counts, red blood cell-diluting pipettes were used. The oxalated blood was diluted 1 to 100 with Earle's solution (Earle, W. R., 1943, J. Nat. Cancer Inst. 4: 165–212) which had been cooled to 3 C.

Earle's solution was suggested (by R. C. S.) as a diluting medium after we had failed with the usual diluting fluids and with fish saline solution. *Cryptobia* failed to survive more than a few seconds when fish saline (0.9 percent NaCl) at room temperature was used as the diluting fluid. When the saline was cooled to 3 C, the hemoflagellates survived for about 1 minute. In Earle's solution cooled to 3 C, the hemoflagellates lived for about 15 minutes.

Counts of the living *Cryptobia* were made in an improved Neubauer hemocytometer using the same counting techniques employed in counting mammalian leucocytes, and the number per cubic millimeter of blood was then calculated. These data are compared with the relative abundance of *Cryptobia* on dried blood smears taken from the same fish (table I).

TABLE I. *The estimated numbers of* Cryptobia *in the blood of infected coho salmon, compared to their relative abundance on stained dried smears.*

Fish	Date	Sex	Estimated number per cu mm of blood	Relative abundance on smears
1	1–16–61	M	2375	++++
2	"	M	5750	++++
3	"	M	250	+
4	"	M	875	++++
5	"	F	0	++
6	"	F	625	++++
7	"	M	250	++
8	"	F	875	+++
9	"	F	500	++
10	"	F	250	++
11	1–24–61	F	125	+
12	"	M	5625	+++
13	"	M	0	0
14	"	F	7750	++++

These data indicate that the blood of observed infected salmon contained between 125 and 7750 *Cryptobia* per cubic millimeter. There is a rough correlation between the estimate of relative abundance made from dried smears and counts made in the hemocytometer. The data also suggest that (*note* fish 5) a careful examination of the dried smear is perhaps a better criterion for determining the presence of these flagellates in fish with light infections than reliance only on the observation of *Cryptobia* in the chamber of the hemocytometer.

The ability to make counts of these organisms in the blood of fish has provided a technique which can be used in some contemplated studies even though errors may be made in the case of fish with very light infections. No fish should be judged uninfected until a careful search is made of a dried stained blood smear from the animal concerned.

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EFFECT OF HIGH TEMPERATURE ON THE INFECTIVE LARVAE OF *OESOPHAGOSTOMUM COLUMBIANUM* CURTICE, 1890

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The nodular worm *Oesophagostomum* is commonly found in the large intestine of herbivorous animals. In India abou 60 percent of the sheep and cattle are infected with this worm. The morphology of a large number of species of *Oesophagostomum* has been worked out. The eggs pass out with the faeces and hatch outside the host body. The free-living infective larvae are subjected to great variations under different environmental conditions. Both the micro- and macro-environments influence the longevity and morphological features of these infective larvae.

Goodey (1924), while describing the anatomy and biology of *O. dentatum* from the pig, stated that the rhabditiform larvae of *O. dentatum* resembled the larvae of *O. apiostomum* and *O. columbianum* in morphological characters. The larvae of these three species were found to be positively geotropic and resistant to desiccation for 1 or 2 days and exhibited no marked positive thermotropism.

Spindler (1936) reported that the infective larvae of the swine nodular worm *O. dentatum* lived for 14 months on outdoor experimental plots under a heavy growth of grass while those on bare experimental plots survived for approximately 9 months.

Anantaraman (1942) found that the infective larvae of *O. radiatum* were somewhat short-lived, and larvae stored in water and exposed to bright light were alive for nearly 2 months, and those that were protected by a black paper died in 3 months.

MATERIALS AND METHODS

Twenty sheep guts were dissected every week in the Department of Zoology, University of Lucknow, India, for a period of about 2 years. Sixty percent of the sheep were found infected with *Oesophagostomum columbianum*. The parasitic females were picked out of the intestinal contents, washed, and kept in dishes in normal saline at 34 C for about 3 hours. A large number of eggs was obtained in this manner. Petri dish cultures were prepared from these eggs in a medium of cow's feces sterilized at 15 pounds pressure, dried and ground, and mixed with animal charcoal. Filter paper was used in the petri dish lid and moistened every day in order to keep the humidity 100 percent.

Cultures were also made on sterilized nutrient agar plates. Nutrient agar in concentration of 2.8 per 100 cc. was sterilized in 250 ml. flasks stoppered with cotton plug and autoclaved for 15 minutes. This sterilized nutrient agar was poured in 2-inch petri dishes and then kept at freezing temperature. The agar dishes were taken out of the freezing temperature and incubated at 37 C for 4 to 5 hours. Later on the agar dishes were inoculated with a bacterial strain of *Escherichia coli* obtained through the courtesy of the Head of the Pathology Department, Medical College, University of Lucknow, and incubated at 37 C for 24 hours for developing the bacterial colony before they were utilized for preparing cultures. Only the eggs laid in saline solution by the parasitic females of *O. columbianum* were used for these experiments in order to avoid contamination by other sheep nematode eggs.

Observations were made on fresh specimens in Lugol's iodine solution, and on specimens fixed in hot formol alcohol, cleared in lactophenol and mounted in Brandt's glycerine jelly.

RESULTS

Size variations in infective larvae due to temperature. Cultures were prepared from the same batch of eggs laid by the adult females of *O. columbianum* in saline solution, and incubated at temperatures of 25 C, 30 C, 34 C, and 37 C. The optimum temperature for the development of infective larvae was found to be 34 C, and this temperature was used as the control temperature. Individual cultures were removed from the incubators at specific intervals. Cultures with sterilized cow's feces and charcoal were "baermanned" for 1.5 hours and then infective larvae collected in about 25 milliliters of water. Infective larvae from nutrient agar dishes were collected by adding 20 milliliters of warm water to each petri dish, and

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leaving it for 15 to 20 minutes. All infective larvae travel upwards in the water, and are easily taken out of the cultures by decanting.

The maximum number of infective larvae was obtained in 115 hours at 25 C, 95 hours at 30 C, and 82 hours at 34 C. At 37 C the development was rapid, taking only 74 hours, but the percentage of recovery was comparatively very low.

The infective larvae obtained at different temperatures showed size variations with regard to their different structures. The maximum, minimum, and average measurements of 25 infective larvae taken out from each of these cultures at different temperatures mentioned above are recorded in tables I and II. Measurements of both fresh and fixed specimens were taken to give a comparative picture.

TABLE 1. *Measurements in fresh condition of infective larvae of* Oesophagostomum columbianum *at different temperatures (Measurements in mm)*

Temperature		25 C	30 C	34 C	37 C
Total length	Min	.800	.825	.875	.775
	Max	.850	.925	.950	.925
	Av	.822	.870	.905	.842
Total breadth	Min	.027	.030	.027	.027
	Max	.034	.034	.028	.037
	Av	.028	.029	.027	.031
Length of oesophagus	Min	.129	.139	.139	.119
	Max	.156	.177	.166	.166
	Av	.139	.149	.150	.140
Breadth of oesophagus	Min	.013	.013	.013	.013
	Max	.014	.017	.017	.017
	Av	.013	.015	.013	.013
Tail length	Min	.205	.212	.208	.194
	Max	.239	.242	.259	.218
	Av	.230	.226	.228	.202
Excretory pore from ant./end	Min	.116	.119	.109	.119
	Max	.133	.133	.126	.146
	Av	.119	.123	.122	.127

The data obtained in the above experiments have been compared with those of infective larvae described by Veglia (1924), Monnig (1931), Dikmans and Andrews (1933) (table III).

TABLE II. *Measurements after fixation of infective larvae of* Oesophagostomum columbianum *at different temperatures. (Measurements in mm)*

Temperature		25 C	30 C	34 C	37 C
Total length	Min	.800	.775	.825	.775
	Max	.875	.912	.937	.850
	Av	.827	.873	.893	.831
Total breadth	Min	.027	.027	.027	.027
	Max	.028	.034	.028	.034
	Av	.027	.029	.027	.030
Length of oesophagus	Min	.153	.129	.149	.143
	Max	.160	.171	.163	.166
	Av	.155	.153	.157	.159
Breadth of oesophagus	Min	.010	.010	.010	.010
	Max	.014	.017	.014	.013
	Av	.012	.013	.012	.012
Tail length	Min	.205	.205	.208	.177
	Max	.239	.222	.242	.198
	Av	.217	.213	.215	.186
Excretory pore from ant./end	Min	.109	.119	.112	.119
	Max	.119	.129	.136	.129
	Av	.117	.122	.126	.123

From a review of tables I, and II, and III, it is clear that there is a significant variation in the sizes of different structures of the infective larvae of *O. columbianum* developed at different temperatures. The significance becomes more prominent when these measurements are statistically analyzed. For example, in table I the total length of the infective larvae as compared with that at the optimum temperature of 34 C decreases at 25 C, 30 C, and 37 C by 9.17 percent, 3.867 percent and 6.961 percent, respectively, and the total breadth of the infective larvae increases at the above suboptimum temperatures by 3.704 percent, 7.407 percent and 14.813 percent, respectively. The other measurements like the length and breadth of the oesophagus, tail length, and the distance of the excretory pore from the anterior end also show a variation when compared with similar measurements at the optimum temperature.

TABLE III. *Comparative measurements of infective larvae of* Oesophagostomum columbianum *studied by different authors. (Measurements in microns).*

		Total length	Length of oesophagus	Intestinal cell	Ant. end to genital primordium	Larval tail	Anus to tip of tail sheath
Veglia (1924)		796	168		378	80	210
Monnig (1931)	Min	791	157		337	74	204
	Max	840	172		360		235
	Av	830	165		345		223
Dikmans and Andrews (1933)	Min	771	150	60 long	272	60	193
	Max	923	170	12 wide	410	80	235
	Av	840	159		365	71	213
Present authors	Min	875	139	47 × 13	372		208
	Max	950	166	71 × 13	441		259
	Av	905	150	57 × 13	401		228

Longevity of infective larvae at room temperature. Six covered petri dishes, each containing 100 infective larvae in water were kept in the laboratory for observing the effects of day

and night temperatures, and also those of light and darkness. Three experiments were performed during the summer from July to September, and again during winter from November to March, so as to observe the longevity of these larvae under different climatic conditions in a tropical country. It may be mentioned that constant temperature was not maintained in the laboratory where these experiments were conducted.

The variations in temperature in the laboratory were as follows, temperature recorded in C:

	Maximum	Minimum
July	35	31
August	31	30
September	31	27
October	30	26
November	30	19
December	23	16
January	20	16
February	27	20
March	30	25

The percentage of survival of these infective larvae as against the number of days both for summer and winter is plotted in figure 1. In the summer when temperatures are very high, the infective larvae do not survive beyond 50 days, while in winter they could live for as long as 108 days. In summer after 25 days there is a considerable fall in the percentage of survival of the infective larvae; the death rate is high and the larvae decompose quickly. During the winter, the death rate is very gradual and shows no remarkable change at any period.

Longevity of infective larvae at temperatures between 30 C and 50 C. In order to determine the longevity of infective larvae at high temperatures, a known number of infective larvae was placed in separate covered petri dishes containing water and maintained in incubators at temperatures of 30 C, 34 C, 37 C, 40 C, and 45 C. The cultures were kept free from bacterial growth by changing the water frequently. The dishes were examined at intervals of 4 days and the percentage of larvae that survived was recorded. Figure 2 shows this percentage plotted against number of days.

Although 34 C is the optimum temperature for the development of infective larvae, the longest period of survival was 105 days at 30 C. At 37 C, there is a sudden fall in the percentage of survival after 25 days, only 25 percent of the larvae remaining alive. At 40 C only 4 to 5 percent of the larvae could survive up to 12 days. No larvae could survive for more than 4

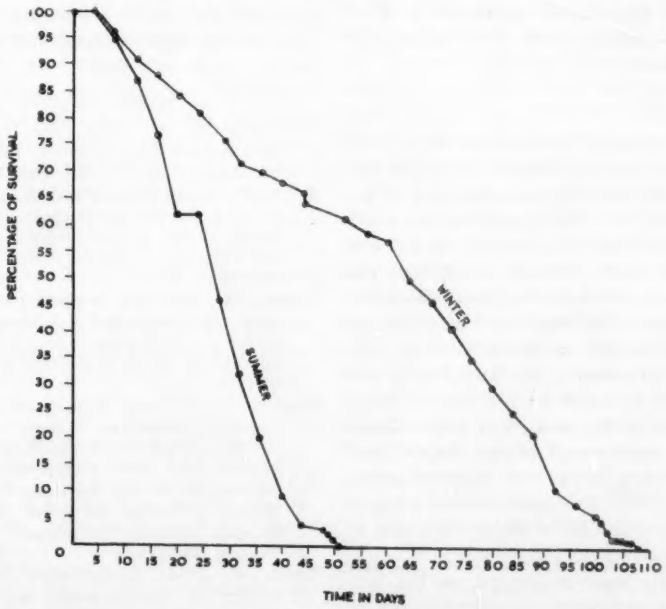

FIGURE 1. Percentage survival of infective larvae of *Oesophagostomum columbianum* at room temperature in summer and winter.

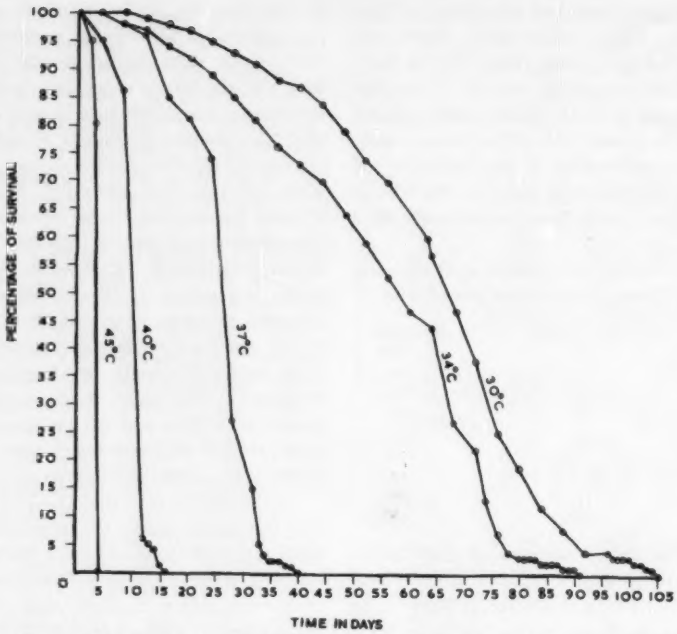

FIGURE 2. Percentage survival of infective larvae of *Oesophagostomum columbianum* at different temperatures.

days at 45 C. Experiments performed at 50 C showed disintegration of the larvae after only 10 to 15 minutes.

SUMMARY

Oesophagostomum columbianum occurs in 60 percent of the sheep at Lucknow. Petri-dish cultures were prepared from the eggs laid by the adult female at 34 C. The infective larvae which developed at different temperatures showed size variations in their different structures. The total length of the infective larvae decreases when developed at suboptimum temperatures, and the total breadth increases at these temperatures as compared to the total length and breadth at the optimum temperature of 34 C.

The effects of day and night temperatures both during summer and winter, on the longevity of infective larvae were observed inside the laboratory. The maximum survival at summer temperatures is for about 50 days and at winter temperatures for 108 days.

Experiments were performed on the longevity of infective larvae at temperatures of 30 C to 50 C. Observations show that there is a maximum survival of 105 days at 30 C and at 50 C larvae could survive only for a few minutes.

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INTESTINAL HELMINTHS IN DOGS AND CATS OF THE BERMUDA ISLANDS AND THEIR POTENTIAL PUBLIC HEALTH SIGNIFICANCE, WITH A REPORT OF A PROBABLE CASE OF VISCERAL LARVA MIGRANS*

ROGER W. WILLIAMS** AND EDWARD L. MENNING***

Various helminth parasites of dogs and cats are known to develop or live in man (*Echinococcus* spp., *Toxocara* spp., *Dipylidium caninum, Ancylostoma braziliense, A. caninum, Uncinaria stenocephala,* and possibly *Trichuris vulpis*) and some are capable of causing very serious pathological conditions. Of the human infections acquired through close association with dogs and cats in the United States, England, Puerto Rico, and undoubtedly other areas, perhaps next to *Echinococcus* the most significant, widespread, and damaging is that known as visceral larva migrans caused by the roundworm of dogs, *Toxocara canis*. The roundworm of the cat, *T. cati,* may also be of some importance in causing this disease in man. Visceral larva migrans is primarily a disease of toddle-age children who ingest soil polluted with the eggs of *Toxocara*. Since *Toxocara* does not mature in man, a paratenic host, no eggs are produced for easy diagnosis. The second stage larvae may migrate through the organ systems (liver, lungs, brain, kidneys, eyes) for several weeks and remain alive encapsulated for many months, sometimes causing *petit mal* attacks, blindness, and even death (Beaver, 1956). Dent (1960) and Ashton (1960) indicate that eye infections have been found following mild *Toxocara* infections in which symptoms were not outstanding. A usual clinical finding, even in the absence of other symptoms, is eosinophilia frequently exceeding 50 percent persisting at a high level usually for a year or more, but which may be absent in eye infections. Dent (1960) states, "Eosinophilia, hepatoplenomegaly, hyperglobulinemia, occurring in a toddler together with a history of pica and close association with a puppy, is almost pathonomonic of the disease." Beaver (1959) points out that there is good reason to suspect *Ancylostoma caninum*, a hookworm of dogs and cats, which persists in the tissues of experimental animals for more than a year, to be involved in visceral larva migrans as well as *Toxocara*.

Various surveys have shown that *Toxocara,* hookworm, and whipworm are widely distributed and in general are more abundant in young animals. The present survey represents an attempt to determine the prevalence of helminth parasite of dogs and cats in the small, geographically isolated area of the Bermuda Islands, and to consider the potential public health significance of the findings as they may influence not only the indigenous population but also the families of American military personnel stationed there and the visiting tourist. Beaver (1959) and Weiner (1960) have emphatically stressed the need for such surveys, particularly of *Toxocara*.

The Bermudas have a land area of about 20 square miles with an estimated human population of approximately 44,000. By law all dogs are required to be registered each year, although many are not. In 1959 more than 4,100 dogs were registered; in 1960, only 2,816. Estimates of the total dog population, from several sources, ranged from 7,000 to 14,000. The true figure is probably between the two. The cat population is thought to be somewhat higher than that of the dog population.

MATERIAL AND METHODS

The standard modified sugar flotation technique was used for concentrating helminth ova from each of 366 stool specimens from dogs and 39 from cats. These animals were pets of military personnel and civilians living scattered throughout the islands. Records were kept of the sex and age of

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all dogs from which specimens were obtained. The age of 1 year was used to separate mature from young dogs. Records were not maintained on the sex of the few cats whose stools were examined.

Egg counts were not routinely made; however, a record was kept of the number of specimens designated as 4+. These stools averaged 10 or more ova per low-power field in the concentrated material. Counts were made on a number of these stools selected at random utilizing the "McMasters Fecal Counting Chamber Kit," using "Sheather's" solution.

A search was made for possible human cases of visceral larva migrans. Two soil samples of 1 sq ft and 1-in deep were taken from the yard where a suspected case of visceral larva migrans played. One sample was taken from a "natural" sandhole where children played, and one from an area at the back steps into which all the water from the backyard ran. Each sample was slowly mixed with "Sheather's" solution for 10 minutes. After settling, the supernatant fluid was decanted, filtered through wet cheese cloth, and centrifuged. Samples taken from the top of the tubes were examined for *Toxocara* eggs.

An autopsy was made on five dogs and the species of all adult hookworms recovered was determined.

RESULTS

The eggs of *Toxascaris leonina*, a roundworm of dogs and cats not known to be pathogenic or to develop in man, were found in only one dog included in the survey, a 6-month-old male which was also parasitized by *Toxocara* and hookworm. The junior author has since seen one other dog in the Bermudas infected with this parasite.

About 38 percent of all dogs examined were infected with *Toxocara*, 45 percent of those under 1 year of age and 20 percent of both males and females over a year of age (table I).

TABLE I. *Helminth Infections in Dogs and Cats of the Bermuda Islands.*

Animals		*Toxocara*		Hookworms		*D. caninum*		*T. vulpis*	
Description	No.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
				Dogs					
Females under 1 yr.	136	57	41.18*	83	61.03	17	12.50	3	2.21
Females over 1 yr.	61	12	19.67	20	32.79*	4	4.92	4	6.60
Males under 1 yr.	124	61	49.19†	77	62.10	11	8.96	1	0.81
Males over 1 yr.	45	9	20.00	18	40.00†	2	4.44	3	6.67
(Total)	366	139	37.98	198	54.10	34	9.29	11	3.01
				Cats					
Under 1 yr.	24	6	25.00	6	25.00	3	12.50	3	12.50
Over 1 yr.	15	1	6.66	5	33.33	2	13.33	1	6.66
(Total)	39	7	17.94	11	27.82	5	12.82	4	10.25

The difference between * and † is not statistically significant in the samples studied.

Hookworm eggs were found in 54 percent of the dogs examined. Of those animals under a year of age, about 62 percent were infected, while 36 percent of those over this age were parasitized. *D. caninum* was found in about 9 percent of all dogs studied and *T. vulpis* in 3 percent. Of all puppies under 8 weeks of age, 66 percent were infected with both *Toxocara* and hookworm (table II).

TABLE II. *Helminth Infections in Dogs of the Bermuda Islands under 8 Weeks of Age.*

Animals		*Toxocara*		Hookworms		*D. caninum*		*T. vulpis*		Negative	
Description	No.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Female dogs	36	23	63.88	25	69.44	3	8.33	1	2.77	6	16.66
Male dogs	35	24	68.57	22	62.85	2	5.71	0	0.00	6	17.14
(Total)	71	47	66.19	47	66.19	5	7.04	1	1.40	12	16.90

Of all dogs under 1 year of age infected with *Toxocara*, 23.7 percent were classified as 4+, while none of the dogs over this age fell into this category. Of the dogs under 1 year of age infected with hookworms, 14.4 percent presented 4+ slides as did 5.3 percent of the infected dogs over 1 year of age. All in the latter group were females. The eggs per gam (e.p.g.) counts of four of the 4+ *Toxocara* dog stools taken at random ranged from 14,400 to 63,600. Five stools counted for hookworm eggs ranged from 15,000 to 18,200 e.p.g.

The sample of cats (table I), of which about 18 percent were infected with *Toxocara*, 28 percent with hookworm, 13 percent with *D. caninum* and 10 percent with *vulpis*, is per-

haps too small from which to draw any conclusions other than to say that the infection rates with both *Toxocara* and hookworm appear to be lower than that found by a number of investigators working in various parts of the United States as reported by Dorman and van Ostrand (1958).

Some of the salient aspect of history and clinical features, when considered collectively, which would tend to point to visceral larva migrans in a suspected case were:

a) The suspected case was a 2.5-year-old female (toddler-age).
b) Two dogs lived in the home with her.
c) The child's mother reported that she did eat "a lot of dirt."
d) The child had a capricious appetite.
e) Upon admittance to the hospital bronchiospasmas, wheezes, and fine rales were noted over both lung fields.
f) X-rays showed bronchovascular markings prominent and a rounded density measuring approximately 1 cm in diameter.
g) During the 15 days the child was in the hospital, her white blood cell count dropped from 60,000 to 13,750 and 8 months later was 10,000. Her eosinophile count varied from 50 to 65 percent while in the hospital. Eight months later it was still 21 percent.
h) Numerous viable *Toxocara* eggs were found in the two soil samples collected from the child's play area.

The Air Force pediatrician had diagnosed, to his satisfaction, two cases of visceral larva migrans among the children of United States military personnel stationed in the Bermudas.

All hookworms taken from the five autopsied dogs were *A. caninum*.

DISCUSSION

Dorman and van Ostrand (1958), Ehrenford (1957), Gross and Rebrassier (1922), Hindman and Baker (1936) and Vaughn and Jordan (1960) have studied the incidence of *Toxocara* and other intestinal parasites of dogs in various areas of the United States. The infection rate of *Toxocara* ranged from 2 to 23 percent in these areas. Some of these investigators felt that an infection rate of 7 percent must be considered hazardous to children. The overall infection rate of 38 percent in the Bermudas would, therefore, appear to present a potentially serious condition. Ehrenford (1957) showed that at the time and place of his study male dogs had a much higher incidence of *Toxocara* than did female dogs at all ages, and that little age resistance developed in mature male dogs. Vaughn and Jordan (1960) failed to find any sex difference in their New Orleans study. We likewise failed to find a sex difference in the Bermudas, since the mature males displayed the same rate of infection as did mature females. Thus we found in the Bermuda Islands not only a higher overall incidence of *Toxocara* in dogs than in other areas studied, but also a higher incidence in older dogs (20 percent), which permits of a wider spread of this infection to other dogs and to children. Whatever the potential hazard of visceral larva migrans to children may be in the areas studied in the United States, it must be considered considerably greater in the Bermudas. This is true not only because of the high incidence of *Toxocara* in the Bermuda dogs, but also because of the high incidence of *A. caninum* (53 percent), the larvae of which many also cause visceral larva migrans, and the potential hazard offered by the infected cats.

Another great hazard appears to exist here. A survey made a number of years ago indicated that in certain areas of the Bermudas 85 to 90 percent of the school children were infected with *Ascaris lumbricoides*. Dent (1960) has pointed out that the influence of previously or concomitantly acquired infections with *Ascaris* on the hypersensitive or "immune state" of an individual suffering from visceral larva migrans is not known but that young children sensitized by even minimal numbers of *Toxocara* and who are asympotomatic may when subjected to reinfection with *Toxocara* or *Ascaris* suddenly develop severe and occasionally fatal bronchial asthma.

It should be emphasized, as others have done, that the occurrence of *Ascaris* ova in the stools does not rule out *Toxocara* infection, but suggests that the individual had ingested "dirt," and perhaps with it *Toxacara* ova.

Thus the potential hazard to small children playing in areas where dogs are permitted to roam in the Bermuda Islands cannot be minimized. Consideration, by physicians and veterinarians alike, should be given to constructive measures for preventing transmission and hu-

man infection with *Toxocara*. Sprent and English (1958) and Gibson (1960) outline such measures.

Danger of human infection with other intestinal helminths of dogs and cats seems to be minimal in the Bermuda Islands. According to local physicians, "creeping erruption," usually caused by the hookworm *A. barsiliense*, does not occur there, probably because *A. caninum* may be the only hookworm parasitizing dogs and cats as indicated in our autopsy findings.

Although 9 percent of all dog stools examined contained egg packets of the sectode *D. caninum*, the sugar flotation method is not a particularly good technique for the isolation of cestode ova. Further, because of the rather sporadic output of segments containing egg capsules, only about 40 percent of the infected animals are likely to be found infected by a single stool examination; therefore, the actual incidence may approximate 25 percent. Belding (1958) reports that this parasite is found in at least 50 percent of the dogs in the United States. According to Moore and Connell (1960, 1961), only 14 human infections with *D. caninum* have been reported from the United States. It would appear unlikely that a probable infection rate of less than 50 percent in the dogs of the Bermudas would produce more human infections than in the United States and may, therefore, be considered of doubtful public health significance there. The whipworm infection rate of 3 percent is lower than that reported by other investigators in other surveys and would also appear to be of little public health significance in the Bermudas.

SUMMARY

Approximately 45 percent of 260 dogs under one year of age and 20 percent of 106 dogs over this age (38 percent of all dogs) were found to be infected with the roundworm *Toxocara canis* in the Bermuda Islands. *Toxascaris leonina* was found in only one animal. *Ancylostoma caninum* may be the only hookworm of dogs in the study area, as indicated by autopsy findings, and, therefore, "creeping eruption" is seldom if ever seen. Over 61 percent of the young dogs and 35 percent of those over one year of age (54 percent of all dogs) were parasitized by this hookworm. There was no significant difference between the infection rate in the two sexes with either of these parasites in the two age groups. Cats also had a high rate of infection with *Toxocara* and *A. caninum*, but not as high as in most areas where surveys have been conducted in the United States.

Since *Toxocara* is known to cause visceral larva migrans in toddler-age children, and *A. caninum* may also, it is evident that the potential for this disease in the Bermuda Islands is significant and considerably greater than in any area so far studied in the United States. The residents of these islands need to be educated to the dangers of close association with household pets to their children.

Some of the history and clinical findings are presented of a probable case of visceral larva migrans in a 2.5-year-old "dirt-eating" child in whose play area numerous viable *Toxocara* eggs were recovered.

Dipylidium caninum and *Trichuris vulpis*, also found in dogs and cats in the Bermudas, are probably of little public health importance there.

ACKNOWLEDGMENTS

Thanks are due to Dr. Audrey I. Penesten for working space in her laboratory and the use of her list of dog and cat owners, and to Dr. R. E. Shaw, pathologist at the King Edward VII Memorial Hospital, for his kind assistance in obtaining clinical information on the probable case of visceral larva migrans.

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RESEARCH NOTE

NEMATODE AFFECTING THE AORTA IN INDIAN CAPRINE AND EQUINE HOSTS.

Bhatia and Sood (1959, Curr. Sci. **28**: 490–491) reported in aortic affections of local goats (*Capra hircus*) the occurrence of 4th- and 5th-stage juveniles of a filarial worm. It was encountered during four autopsies and identified provisionally as belonging to a species of *Tetrapetalonema* Faust, 1935. A brief account of the lesion was also given. A similar infestation was found in a Hill-goat (*C. sibirica*), examined at Pithoragarh (Tibetan border Hill district in this State) and later in two ponies (*Equus caballus orientalis*) and a female donkey (*Equus asinus*), both locally.

The large number of immature males and females had intestines pinkish in colour and filled with blood due to feeding habits. Whether the assignment of the specimens to the highly confused genus *Tetrapetalonema* was correct, or the four different host species yielding fully identical specimens without structural variation were all accidental (arising out of the ingestion of the infective intermediate hosts of the well known spiruroid, *Spirocerca lupi* (Rud., 1809) Chitwood, 1933, a common parasite of carnivores and widespread in various parts of this country) needed clarification and confirmation. Gupta (1961, M.V.Sc. Thesis, Agra Univ., unpublished), working on some of the parasitic worms from carnivores, examined the juvenile stages collected from the aortic lesions in dogs and also recovered the earlier 3d-stage from the liver.

No recognizable differences appear between the immature forms available for study from the canine and other hosts, there being complete agreement in the number and arrangement of head papillae, shape of buccal capsule, position of excretory pore and nerve-ring, and character of external genitalia (caudal alae, preanal and post-anal papillae and spicules in males), and position of vulva and extent of muscular vagina in females. Further, examination of stained serial sections of the lesions from the different host species has provided additional support from histological details of the stages cut. Evidently, the aortic infestation is of *S. lupi* which, it is believed, had earlier been reported from *E. asinus* by Seurat (1916—reference not traceable) and our report in the first record of the species in goats and horse. Only experimental work, however, by feeding the infective stages to these herbivorous animals, could confirm these findings.

The lesions in the bulbous aorta and the initial region of the thoracic aorta yielding the specimens had extended to the branching arteries. One or two worms could be collected from such a location. Unlike the pathological changes exhibited in dogs, the differentsized nodules, though uneven in character, were with prominent ulceration and haemorrhagic spots containing blood clots around the partly embedded worms. The intimal coat was greatly thickened and internally showed calcareous deposits along with black blood pigments. Apparently, it was the internal coat that had suffered more from the continued impingement of these stages and

the degree of severity of the lesion caused by the arrested forms appeared to depend upon the number harbored and the comparative thickness of the aortic wall. Serially cut and stained sections revealed the worms inside tunnels with surrounding blood clots and blood cells and pigment deposited in the intestinal mucosa along with great disruption of the tunica intima showing a heavy lymphocytic infiltration (fig. 1). A similar condition with polymorphonuclear leucocytes, lymphocytes, eosinophiles and a few plasma cells, the first three predominating, and a distinct development of fibrous tissue were observed at sites where anterior ends were embedded (fig. 2).

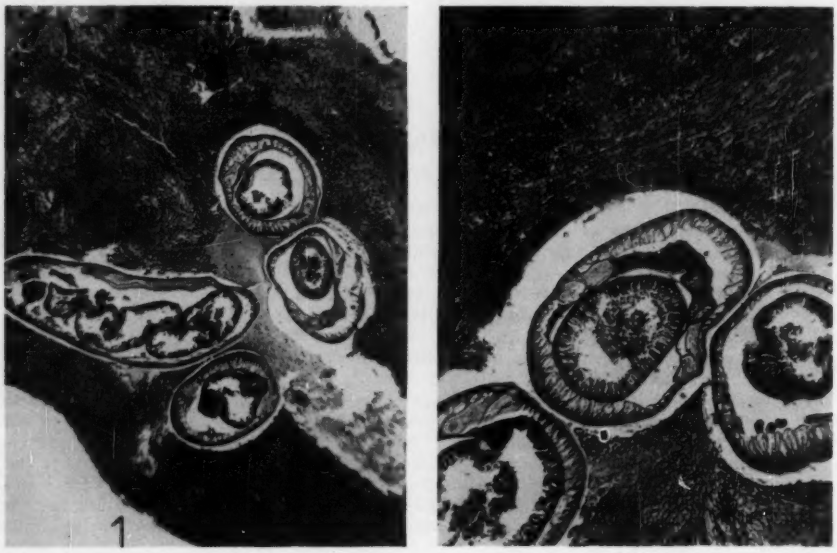

FIGURE 1. Photomicrograph of section of donkey's aorta showing worm cut in different planes inside the aortic wall. × 35.
FIGURE 2. Photomicrograph of section of donkey's aorta showing reaction around the worm. × 70.

This report points to the possibility of a greater incidence of such cases. In consequence, there is danger of the common oesophageal tumor worm of the dog acting as a potential pathogen in domestic animals during its development, particularly in areas where animal husbandry practices are not yet executed on sound scientific lines.

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MOLINEUS VEXILLARIUS SP. N. (NEMATODA: TRICHOSTRONGYLIDAE) FROM A PERUVIAN PRIMATE, *TAMARINUS NIGRICOLLIS* (SPIX, 1823)*

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Fifty-one specimens of a new species of *Molineus* were recovered from two of seven Peruvian tamarins examined in the course of a survey of certain South American primates for ecto- and endoparasites. *Tamarinus nigricollis* is a small marmoset-like primate found only in a belt of Amazon forest lowlands stretching southwest from Pebas and Iquitos parallel to the Ucayali River in eastern Peru (Osman Hill, 1957). The animals were captured in this region and shipped by air, via Lima, to San Francisco in January 1961. Six of the seven studied died a few days after arrival and were examined soon after death; the seventh animal was sacrificed and examined immediately. Two specimens were removed from the duodenum of one of the animals, 49 from the stomach and duodenum of another. The other five tamarins did not harbor this parasite. A variety of other helminths were found in the seven animals, including acanthocephalans (in four), cestodes (in two), oxyurids (in three), and metastrongyles (in one). The trichostrongyles were fixed in hot 70 percent alcohol, and preserved in 70 percent alcohol plus 5 percent glycerine or mounted in glycerine jelly.

Molineus vexillarius sp. n.

Host. Tamarin, sagui, *Tamarinus nigricollis* (Spix, 1823).

Location. Duodenum and pyloric portion of stomach.

Locality. Amazon lowlands of eastern Peru.

Material. Holotype: male, U. S. National Museum, Helminthological Collection No. 59559. Paratypes: 35 females and 15 males. Of these, 3 males and 3 females deposited in U. S. National Museum, Helminthological Collection No. 59560.

Description. Minute slender worms, pale red in life. Cuticle, except for cephalic portion, with 10 to 12 longitudinal lines and fine transverse striations. Cephalic cuticle inflated, with some variation in extent of inflation and position of maximal inflation (all specimens fixed and studied in same manner). Cephalic cuticle bears 17 transverse striae. First striation about 12 microns from anterior end of head. Second striation 4 microns posterior to first. Remaining striae at 2-micron intervals. First and second striae more prominent than others. No cephalic papillae. Shallow buccal cavity. Short post-cephalic cuticular inflations in a few specimens. Esophagus clavate. Excretory pore prominent, sometimes with lips inflated, at same level as cervical groove. Cervical papillae present, prominent or inconspicuous. Nerve ring located anterior to cervical groove.

Male. Wide bursa with large lateral lobes and small dorsal lobe. No prebursal papillae. Between ventral and lateral rays inner surface of lateral lobes covered by very small spinelets, giving membrane granular appearance. Ventral rays of equal length and thickness, diverging distally and reaching margin of bursa. Antero-lateral ray relatively long, about two-thirds length of other rays, diverging from ventral and lateral rays near its base. Antero-lateral ray does not reach edge of bursa. Medio-lateral and postero-lateral rays equal, reaching bursa margin, and diverging only at tips. Externo-dorsal rays, equal in thickness to other rays, arise from common trunk with dorsal ray and do not reach margin of bursa. Dorsal ray small, bifurcating distally. Each primary branch splits distally to form a longer curved lateral branch and a smaller medial branch which divides into two short prongs. Spicules equal, complex, and distinctive in form. Proximal end of spicule blunt rounded hook surmounting broad plate crossed by single diagonal ridge. Distally spicule branches into terminally-barbed medial process, and mediolateral and lateral processes ending posterior to medial process. Mediolateral process consists of a single simple terminally-pointed rod. Lateral process, formed by fusion of two slender rods, terminates in a blunt point which touches but apparently does not fuse with point of medio-lateral process. Gubernaculum slender with proximal linear markings; distal third bent ventrally.

Measurements of males (average, followed by range, with number of measurements in parenthe-

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ses): length, 3.3 mm, 3.0 to 4.0 mm (14); maximum diameter, 67 microns, 50 to 86 microns (14); anterior end to excretory pore, 180 microns, 156 to 195 microns (14); anterior end to cervical papillae, 207 microns, 190 to 218 microns (8); length of esophagus, 313 microns, 286 to 353 microns (13); length of cephalic inflation, 43 microns, 40 to 46 microns (15); maximum diameter of cephalic inflation, 29 microns, 24 to 38 microns (15); length of gubernaculum, 63 microns, 54 to 69 microns (13); length of spicules, 111 microns, 102 to 122 microns (13).

Measurements of holotype: length 3.5 mm, maximum diameter 79 microns, anterior end to excretory pore 182 microns, anterior end to cervical papillae 210 microns, length of esophagus 324 microns, length of cephalic inflation 45 microns, maximum diameter of cephalic inflation 30 microns, length of gubernaculum 66 microns, length of spicules 114 microns.

Female. Vulva salient, located about 80 percent of body length from anterior end. Divergent branches of the short sturdy ovejector approximately equal. Length between two sphincters 0.2 to 0.3 mm. Anterior branch of divergent uteri contains 7 to 12 mature ellipsoid ova in a single row, 40 to 52×20 to 29 microns in size. Posterior branch contains 4 to 9 mature ova of similar size. Anus situated less than one-fiftieth of body length from posterior end. Tail tapers to a bluntly trilobed point. From dorsal lobe projects a delicate sharp spine about 13 microns long (8 to 17 microns). Two ventral lobes smaller than dorsal lobe. Final 13 to 18 microns of tail covered by delicate membrane, more or less inflated. Membrane granular in appearance and free of transverse striae when seen fully inflated.

Measurements of females (average, followed by range, with number of measurements in parentheses): length, 3.6 mm, 3.2 to 4.2 mm (30); maximum diameter, 79 microns, 59 to 95 microns (30); vulva to posterior end, 730 microns, 620 to 860 microns (32); anus to posterior end, 69 microns, 56 to 80 microns (30); anterior end to excretory pore, 185 microns, 166 to 208 microns (33); anterior end to cervical papillae, 207 microns, 182 to 234 (22); length of esophagus, 347 microns, 300 to 403 microns (28); maximum diameter of esophagus, 31 microns, 26 to 36 microns (26); length of cephalic inflation, 44 microns, 36 to 48 microns (33); maximum diameter of cephalic inflation, 29 microns, 20 to 36 microns (33).

Diagnosis. Trichostrongylid nematode of the genus *Molineus*, parasitic in the duodenum and stomach of a primate. Differs from all other species of the genus except *M. congolensis*, *M. nasuae*, *M. torulosus* and *M. elegans*, by possession of barb on one process of male spicule. *M. congolensis* males and females are much larger intrahepatic parasites of African rodents, differing also in having more longitudinal lines (16), more transverse cephalic striae (24), and dorsal ray without distinct primary bifurcation (terminal branches arise directly from dorsal trunk). *M. nasuae*, a parasite of the coati, differs in having fewer transverse cephalic striae (less than 12), relatively short antero-lateral rays, a simple conical female tail, often curved ventrally, and a dorsal ray with terminal branches arising immediately below the primary bifurcation. *M. torulosus*, parasitic in *Cebus* and *Saimiri* monkeys, is two to three times longer (females 10.1 to 12.0 mm; males 8.2 to 9.0 mm) than *M. vexillarius*, and differs also in having more longitudinal lines (approximately 14), relatively short antero-lateral rays, and a simple conical, terminally truncated, female tail. In addition, *M. torulosus* spicules differ in several minor respects from those of *M. vexillarius*, and externo-dorsal rays arise directly from base of common dorsal trunk while those of *M. vexillarius* arise more distally on the trunk (Cameron, 1923; Skrjabin et al, 1954). *M. elegans*, closest to *M. vexillarius*, is also found in South American primates (*Saimiri sciurea* of eastern Brazil) (Travassos, 1937). The two species differ as follows: (1) dorsal ray: in *M. elegans* terminal branching occurs immediately below the primary bifurcation while in *M. vexillarius* terminal branching occurs from relatively long trunks formed by the primary bifurcation; (2) *M. elegans* externo-dorsal rays arise directly from base of common dorsal trunk while those of *M. vexillarius* arise more distally on the trunk; (3) antero-lateral ray of *M. elegans* relatively shorter; (4) minor differences in spicule morphology; (5) *M. vexillarius* gubernaculum and spicules considerably shorter than those of *M. elegans*, although overall length of the males is approximately the same. Gubernaculum: 54 to 69 microns (*M. vexillarius*) vs 71 to 78 microns (*M. elegans*); Spicules: 102 to 122 microns (*M. vexillarius*) vs 120 to 134 microns (*M. elegans*); (6) *M. elegans* females 20 to 25 percent longer; (7) female tail simple in *M. elegans*, lobed (usually trilobate) in *M. vexillarius*.

DISCUSSION

This species brings the number presently assigned to the genus *Molineus* to fifteen (Skrjabin et al, 1954). Two, *M. vogelianus* De Muro, 1933, and *M. congolensis* Adam and Wanson, 1954, are African species; two are Asian, *M. planicipitis* (Cameron, 1928) Travasso, 1937, and *M. asiaticus* Tubangui and Masilungan, 1938; and one, *M. patens* (Dujardin, 1845) Petrov, 1928, occurs in Europe, the USSR, and North America. The remainder have been reported only from the Western Hemisphere (or in animals originating in this area). In addition to *M. vexillarius* these species are: *M. felineus* Cameron, 1923; *M. barbaris* Cameron, 1936; *M. barbatus* Chandler, 1942; *M. elegans* (Travassos 1921) Travassos and Darriba, 1929; *M. major* Cameron, 1936; *M. nasuae* Lent and Freitas, 1938; *M. paraensis* Travassos, 1937; *M. pardalis* Cameron, 1936;

and *M. torulosus* (Molin, 1861) Cameron, 1923. The present species and *M. vogelianus, M. elegans,* and *M. torulosus* have been recovered from primate hosts; other hosts, with one exception, are carnivores of the Felidae, Mustelidae, and Procyonidae. *M. congolensis* is exceptional in that it was recovered from rodents, and from intrahepatic sites (Adam and Wanson, 1954). All other hosts harbor *Molineus* in the stomach, small intestine, or both.

Known primate hosts of *Molineus* include the African potto, *Perodicticus potto* (with *M. Vogelianus*); the Peruvian tamarin, *Tamarinus nigricollis (M. vexillarius);* the squirrel monkey, *Saimiri sciurea,* from eastern Brazil (*M. elegans, M. torulosus);* and other Brazilian monkey hosts of *M. torulosus: Cebus azarae, Cebus capucinus,* and *Cebus fatuellus.* The closest affinities of *M. vexillarius* are with those species found in other South American primates. It least resembles those species found in unrelated hosts in Asia, Africa, and North America.

M. vexillarius appears to be non-pathogenic in its natural host. The worms were found lying in secretions on the mucosal surfaces, never attached to the bowel wall. There was no evidence of inflammation of the mucosa, even in the first and second portions of the duodenum where the worms were most heavily concentrated. Only a few worms were found in the stomach and third portion of the duodenum.

SUMMARY

A new species, *Molineus vexillarius* (Nematoda: Trichostrongylidae), is described from a small marmoset-like primate of eastern Peru, *Tamarinus nigricollis* (Spix, 1823). It appears to be non-pathogenic in its natural host. This is the first trichostrongylid to be recorded from this host, and the fourth species of the genus to be recorded from a primate.

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PLATE I

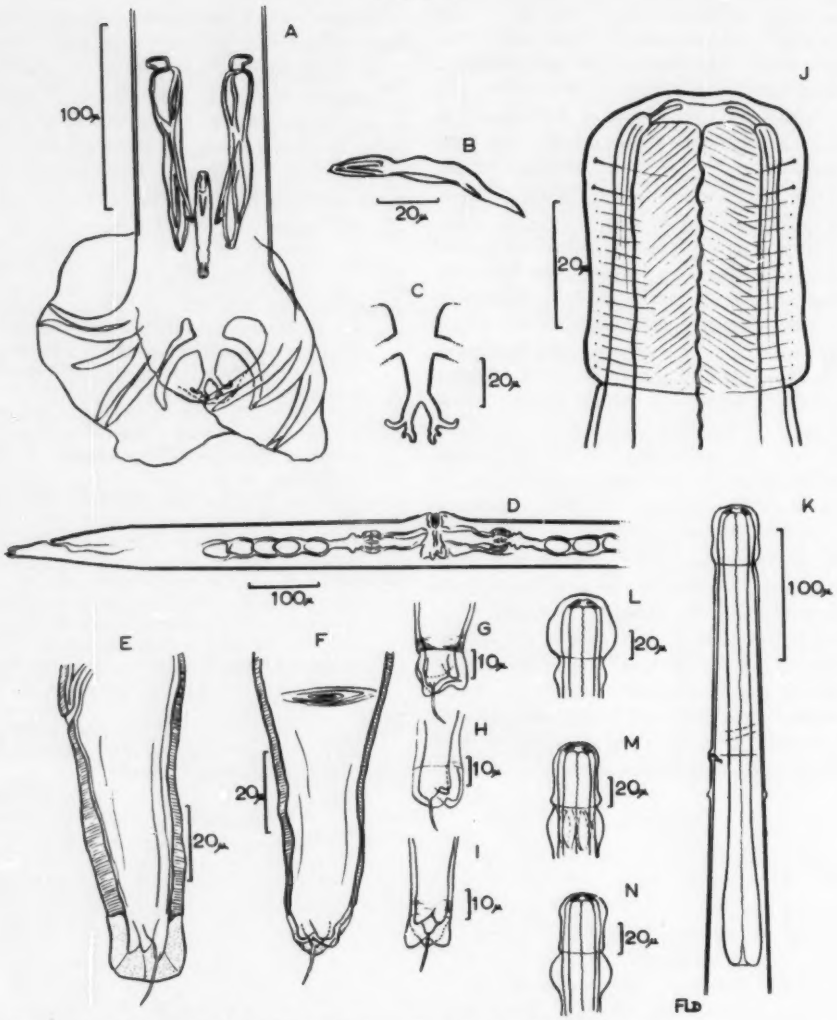

FIGURE A. Male, posterior end, ventral view; length of specimen 4.0 mm.
FIGURE B. Male, gubernaculum, laterial view; 3.4 mm.
FIGURE C. Male, dorsal ray; 4.0 mm.
FIGURE D. Female, posterior end, lateral view; 3.5 mm.
FIGURE E. Female, lateral view of tail with caudal membrane fully inflated; 3.3 mm.
FIGURE F. Female, ventral view of tail with caudal membrane collapsed; 3.9 mm.
FIGURE G., H., I. Females, typical tails: G, dorsal view; H, lateral view; I ventral view.
FIGURE J. Male, typical head; 3.3 mm.
FIGURE K. Male, anterior end; 3.3 mm.
FIGURE L. Female, fully inflated cephalic membrane; 3.5 mm.
FIGURE M. Female, head showing six post-cephalic cuticular inflations and greatest degree of cephalic inflation at posterior end of head; 3.7 mm.
FIGURE N. Female, head showing post-cephalic inflations and greatest degree of cephalic inflation at anterior end of head; 3.6 mm.

EFFECTS OF CROWDING AND OF SUPERINFECTION ON HABITAT SELECTION AND EGG PRODUCTION IN *ANCYLOSTOMA CANINUM**

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Over a period of years a considerable amount of information has accumulated on the host-parasite relationships of the hookworms. Several workers have tried to relate egg counts to the number of worms present. Stoll and Tseng (1925) found that egg counts gave a clue to the severity of hookworm disease in China. They indicated that egg counts, in terms of numbers per gram of feces, were the most satisfactory expression of relative worm burden.

Sarles (1929) and McCoy (1931), working with *Ancylostoma caninum* in experimentally infected dogs, found an inverse relationship between egg production and the number and age of worms, but failed to find a correlation between worm burden and size of worms.

In studies of the effect of superimposed infections on the proportion of worms able to develop, Herrick (1928) found some evidence of increased resistance after two or three infections, but not after one. Experiments by McCoy (1931), Foster (1935) and Otto (1941) on superinfection tend to confirm Herrick's observations. Herrick (1928) and McCoy (1931) also found that the egg output per female in dogs with repeated infections was only one-third that observed in animals with single infections.

The factors responsible for the decrease in number of eggs produced per female under crowded conditions have been considered by Yazima and Machida (1958). The position of the worm in the small intestine and competition for the location offering optimum conditions have been suggested as factors which influence the number of eggs each female can produce. These factors were reexamined in the present study.

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MATERIALS AND METHODS

Short-haired mongrel terriers, 2 to 3 months old at the beginning of the experiment, weighing 6 kg or less at the time of sacrifice, were fed milk in the morning and Purina dog kibblets mixed with cooked beef in the afternoon. All gained weight steadily. They were kept in individual metal cages which were scrubbed daily with hot water to prevent accidental infections. Uninfected and infected dogs were maintained under similar conditions, all being rotated into different cages at weekly intervals. As the stools of the control animals were checked at the same time as were those of the experimental animals and were constantly negative, it is assumed that accidental reinfection of the experimental animals did not occur.

Infective larvae of *A. caninum* obtained from 10-day-old charcoal cultures were collected with moist gauze pads and concentrated by the method of Beaver (1953).

Twelve pups were inoculated by stomach tube with single graded doses of 10 to 2,000 larvae. In the same manner, four were given 3 or 4 doses of larvae at weekly intervals, two (dogs 14, 15) receiving a total of 3,000 larvae and two (dogs 16, 17) a total of 10,000. These four animals are referred to as "superinfected."

Daily estimations of egg production were made by the dilution method of Stoll and Hausheer (1926) and were based on the average of two counts on each sample.

The dogs were sacrificed approximately 8 weeks after inoculation. The intestine was removed, measured, and opened in order to determine the number of adult worms present and their position in the intestine. From these data, the means of the daily egg counts were determined for each week and the average number of eggs produced per female per gram of feces was computed. Also, the proportion of adults developing from each inoculation was calculated and the degree of crowding of the worms was estimated.

RESULTS

The egg production per female worm was correlated with the number of adult worms present and the amount of space available in the small intestine. Under the conditions of this experiment, as the worm burden and the degree of crowding increased, the number of eggs produced per female worm decreased except in dogs

1, 7, and 17 (table I, figs. 1 and 2). The maximum number of eggs produced per female per gram of feces was 286, in a dog harboring only 9 adult worms. However, the rate of egg production did not differ appreciably when less

TABLE 1. *The number of adult hookworms recovered, their egg production and the degree of crowding in the small intestine of dogs infected 2 months previously.*

Dog No.	No. adults recovered	Mean eggs per female per gram	Cm int. per worm*
1	4	190	> 25.0
2	9	286	13.3
7	35	115	4.8
17†	58	149	2.7
3	59	227	3.4
4	69	248	2.2
6	85	203	2.0
5	93	182	2.4
8	158	138	1.3
10	173	139	1.3
9	179	107	1.0
11	214	68	0.7
16†	251	59	1.0
15†	295	57	0.7
14†	335	43	0.8
12	457	11	0.4

* Assuming even distribution of worms throughout the small intestine.
† Repeated infections.

than 200 adults were present. As the number of adults increased above 200 and the available space per worm became less than that within 1 cm length of the intestine, the egg production was depressed. In one animal, in which 457 adults were present, the egg output per female was 11 per gram of feces and the portion of the small intestine's length available for each worm was 0.35 cm (table I).

After the intestine of all 16 dogs had been opened, examined and measured, and the number and position of the worms recorded, it was apparent that in the majority of the animals the jejunum, especially the midportion, was the preferred area if not preoccupied (table II, fig. 3). A greater proportion of adults was observed in this region in 7 of the 11 dogs that had received a single inoculation of 1,000 larvae or less. In the three animals that had received 250, in the one that had received 2,000 and in one (No. 8) of the four animals that had received 1,000 larvae, the adults were most numerous in the ileum. Of the four animals with superinfections, the two given a total of 3,000 larvae had a larger proportion of adults in the ileum; in one (No. 16) of the two given 10,000 larvae, the worms were equally divided between

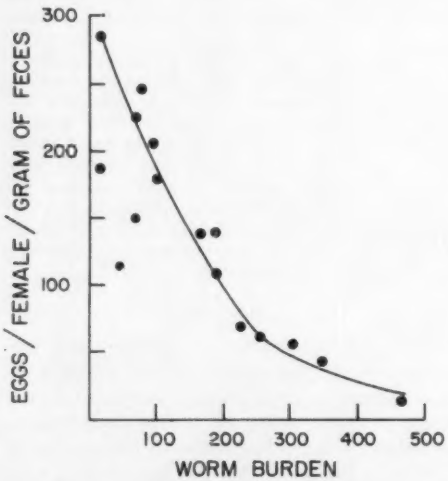

FIGURE 1. Egg output of *Ancylostoma caninum* in relation to worm burden.

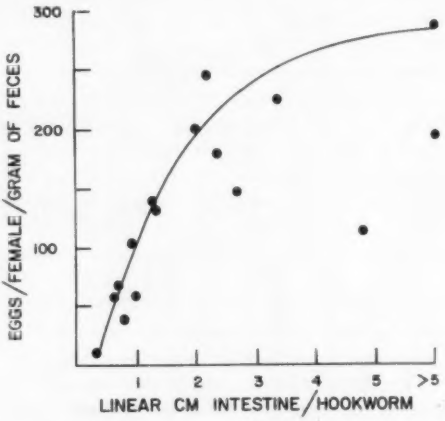

FIGURE 2. Egg output of *Ancylostoma caninum* in relation to the space available in the small intestine. In the two dogs with more than 5 cm of intestine per hookworm, only 4 and 9 worms, respectively, were present.

the jejunum and ileum, and in the other (No. 17) they were more numerous in the jejunum. Worms were found in the duodenum in 11, in the caecum in 5, and in the colon in 7 of the 16 dogs. When worms were present in the caecum and colon, some were always in the duodenum also (table II).

The percentage of larvae becoming adults in the small intestine and the spatial relationship of these worms to each other are recorded in

TABLE II. *The number of adult worms recovered from, and the distribution of these adults in, the intestine of dogs infected 2 months previously with graded doses of larvae.*

Dog No.	No. larvae administered	No. adults recovered	Distribution of adults in intestine*				
			Duodenum	Jejunum	Ileum	Caecum	Colon
1	10	4	0	100	0	0	0
2	10	9	0	77	23	0	0
3	100	59	5	78	17	0	0
4	100	69	7	55	38	0	0
5	250	93	1	20	71	3	5
6	250	85	0	47	53	0	0
7	250	35	26	23	51	0	0
8	1,000	158	0	16	84	0	0
9	1,000	179	2	65	32	0	0
10	1,000	173	0	65	35	0	0
11	1,000	214	17	46	30	0	7
12	2,000	457	6	34	60	0	< 1
14†	3,000	335	7	22	65	5	1
15†	3,000	295	2	36	60	1	1
16†	10,000	251	15	42	40	< 1	3
17†	10,000	58	5	60	30	3	2

* Expressed as per cent of total number of adults.
† Repeated infections.

table III. In animals inoculated with 10 larvae, an average of 65 percent established themselves in the intestine and with more than 13 cm of the intestine available per worm. In animals with 100 larvae, an average of 64 percent of the larvae developed with approximately 2.8 cm available per worm; with 250, 1,000 and 2,000 larvae, an average of 27, 17 and 23 percent, respectively, developed and the corresponding portions of the intestine available per worm were 3.1, 1.1 and 0.35 cm. In the superinfected animals given 3,000 and 10,000 larvae, 10.5 percent and 1.5 percent, respectively, developed into adults with 0.74 and 1.8 cm per worm

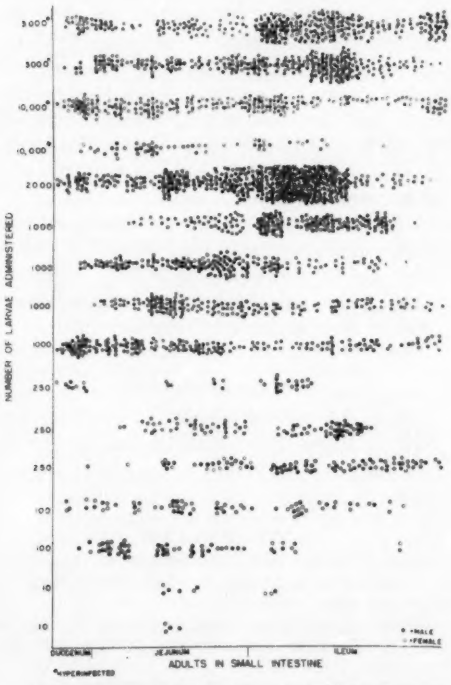

FIGURE 3. Distribution of adult hookworms in the small intestine of dogs inoculated 8 weeks previously with graded numbers of larvae.

TABLE III. *The percentage of adult hookworms developed and their spacing and position in the intestine of dogs inoculated 2 months previously.*

Dog No.	No. larvae inoculated	Pct. larvae recovered as adults	Linear cm per worm*			
			Duo.	Jej.	Ileum	S. int.
1	10	40	—	25.0	—	> 25.0
2	10	90	—	8.6	30.0	13.3
3	100	59	7.7	1.9	8.5	3.4
4	100	69	5.0	1.5	2.7	2.2
5	250	34	25.0	3.4	1.8	2.4
6	250	30	—	1.8	2.0	2.0
7	250	14	2.8	6.6	5.0	4.8
8	1,000	16	—	3.2	0.8	1.3
9	1,000	18	3.8	0.6	1.6	1.0
10	1,000	17	—	0.7	2.3	1.3
11	1,000	21	0.6	0.5	1.2	0.7
12	2,000	23	0.7	0.4	2.7	0.4
14	3,000†	11	1.0	1.5	0.5	0.8
15	3,000†	10	4.2	0.8	0.5	0.7
16	10,000†	2	0.8	0.9	1.2	1.0
17	10,000†	< 1	7.7	1.3	5.0	2.7

* Assuming even distribution of worms present.
† Total larvae used in repeated inoculation.

available in the small intestine. In deriving these approximations of available space, an even distribution of worms throughout the small intestine was assumed. However, observations on the actual distribution of worms in each part of the small intestine revealed a greater degree of crowding in the jejunum in 10, in the ileum in 4 and in the duodenum in 2 of the 16 animals studied (fig. 3).

It is apparent that when the number of larvae inoculated exceeded 100, the percentage of adults developing from the larvae decreased. In the superinfected animals fewer larvae developed into adults than in the case of animals with single infections, even though, theoretically, space was available for a greater number of worms.

DISCUSSION

All of the animals followed the same pattern in regard to percentage of adult worms developing from larvae, egg production, and distribution of worms in the intestine, with the exception of dogs 1, 7, and 17. The comparatively low egg-count obtained for dog 1 can be explained by the fact that only two female worms were present in this animal; as the dilution factor for the Stoll method is 200, the error in low estimates is relatively great.

These dogs had values in the same range as those of dogs 3 and 4 in regard to number of centimeters available per worm and worm burden; yet egg production was less than expected. There was no unusual distribution of worms in the intestine. In dog 7, half of the worms were found in the ileum and the other half were equally distributed between the duodenum and jejunum; in dog 17, approximately 5 percent of the worms were in the duodenum, 60 percent in the jejunum and 30 percent in the ileum. One might speculate that in the latter animal the repeated inoculations stimulated an immune response that inhibited colonization by many worms and depressed the egg production of those that did colonize. However, there is no evidence to support this supposition in view of the fact that dog 7, which was not superinfected, reacted in the same manner, and the 3 other animals (14, 15, and 16) that were superinfected had values comparable to those of the animals with single infections.

No correlation could be found between egg production and the concentration of worms in a particular section of the intestine. The rates of egg production when worms were concentrated in the jejunum or in the ileum were approximately the same. Egg counts in terms of eggs per female per gram of feces appeared to be essentially the same for all infections of less than 200 worms per dog.

SUMMARY

Twelve dogs were infected with single graded doses of *Ancylostoma caninum* infective larvae and four were superinfected with four graded doses of larvae per os given at weekly intervals. The egg production was determined daily and, approximately 2 months after inoculation, the number of adults and their position in the intestine were observed.

The data collected indicated that, both in the superinfected animals and in those with single infections, as the worm burden and the consequent degree of crowding increased, the number of eggs produced per female worm decreased. Superinfection apparently had no influence on the rate of egg output. A greater proportion of adults was found in the jejunum in nine of the animals and in the ileum in the remaining seven. However, the most densely populated area was the duodenum in 2 of the animals, the jejunum in 10, and the ileum in 4. The concentration of worms in a particular region could not be correlated with an increase or decrease in the rate of egg production.

The percentage of larvae developed into adult worms decreased as the number of inoculated larvae increased above 100. Relatively fewer larvae developed into adults in superinfected than in singly infected animals.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Paul C. Beaver for his advice and interest in this study.

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RESEARCH NOTE

RESULTS OF EXAMINATION OF CADAVERS FOR HELMINTH PARASITES.

During the past 13 years the writer has examined 431 cadavers for helminth parasites. The organs examined were the brain, diaphragm, stomach, small intestine, caecum, appendix (when present), liver, and lungs. Examinations of visceral organs were made as dissections by first year medical students progressed. The brains were examined at the time they were being utilized for other purposes. All of the material examined had been preserved by the usual embalming methods except the brains, which were preserved in 10 percent formalin. Most of the subjects had been institutionalized from 1 to 25 years. Two hundred and thirty-one of the 431 subjects were colored males, 168 colored females, 23 white males, and 7 were white females; 2 were Choctaw Indian females.

Lungs and liver were observed for external indications of parasites such as *Echinococcus* and *Paragonimus*. Detailed examinations were made of the lungs as they were being shredded to demonstrate blood supply and divisions of the bronchial tree. Livers were examined in detail as they were being shredded and sectioned to demonstrate the portal circulation and hepatic ducts. Examinations of the contents of the alimentary canal were made at the time these organs were slit longitudinally; representative samples were diluted in water and decanted so as to detect small parasites, such as *Enterobius*. The brains were examined externally and also in section at the time they were being studied for another purpose.

The incidence of *Enterobius vermicularis* was by far the highest, for it was encountered in 122 of the 431 bodies. It was found in the caecum and colon of every infected individual, and in 57 of the appendices examined and in 54 of the small intestines examined. The number of worms recovered from individuals ranged from less than 100 to as many as 12,000 the latter in an adult colored female. The parasites varied in age from too young for the sex to be determined to gravid females.

Ascaris lumbricoides was removed from nine of the bodies examined; eight was the maximum number from one body, that of a colored adult male. All of the ascarids were from the small intestine, except that in one individual they occurred in the large intestine. Recovery at autopsy of 146 adult specimens of *Ascaris lumbricoides* from a 3-year-old colored female, in whom there was complete obstruction as well as perforation of the large intestine, is being included in this report.

Necator americanus was recovered from the small intestine of two colored males; 12 from one subject and macerated parts from another.

Taenia saginata was taken from the small intestine of two colored males.

Degenerate cysticerci of *Echinococcus granulosus* were suspected of being present in the liver of two colored adult males. Histological evidence was insufficient to warrant certain identity.

Trichinella spiralis was recovered from 5 of 100 diaphragms examined.—James W. Ward, *Department of Anatomy, University of Mississippi School of Medicine, Jackson.*

RESEARCH NOTE

SURVIVAL OF *HAEMATOLOECHUS* SP. AND *RHABDIAS BUFONIS* IN ARTIFICIAL MEDIA.

Survival of *Haematoloechus* sp. and *Rhabdias bufonis* in artificial media was studied, using the method previously employed with *Haematoloechus* (Churchill, 1955, J. Parasit. 41: (328–9).

Twelve adult trematodes, removed aseptically from the lungs of 2 *Rana pipiens*, were washed in sterile amphibian Ringer's solution in petri dishes, then transferred to a petri dish (four worms) or test tube (one fluke each) containing Difco Nutrient Agar with an overlay of Ringer's solution. Bits of fresh frog liver were added to the petri dish and to four of the eight tubes.

One tube containing liver was contaminated and the fluke dead on the fourth day. At that time, additional Ringer's was added to each of the other cultures. By the following day bacterial growth was heavy in the remaining liver cultures and those flukes were dead.

On the 18th day more saline solution was added to each of the four surviving cultures. Three days later two tubes showed contamination and the flukes were dead. One additional worm was dead in a tube which still showed no contamination when discarded 3 weeks later.

One trematode survived 40 days. When the experiment began, its ceca had been moderately filled with blood. Saline was added to this tube on the 4th, 18th and 33d days.

At the same time the *Haematoloechus* were isolated, three cultures of *Rhabdias bufonis* from the lungs of the same frogs were made, two in petri dishes and one in a test tube. Liver was omitted from these.

Eggs were laid and nematodes hatched in each. Young worms were present in the test tube culture until the 14th day, but contamination in the petri dishes resulted in death of all the worms by the sixth day.

The one adult *Rhabdias* in the test tube survived 51 days. The last good typical nematode movement seen came on the 44th day. During the next 5 days careful observation was necessary to detect the extremely weak movements but on the 50th day slow, strong, bending movements occurred. The next day the bending was decidedly weaker but still stronger than that seen on the 45th to 49th days. Observations were continued through the 54th day, although there was no evidence of life on the 52d day. As in the case of the *Haematoloechus* culture, saline had been added to the original tube on the 4th, 18th and 33d days.—HELEN M. CHURCHILL AND HONOR CROWTHER, HOLLINS COLLEGE, VIRGINIA.

RESEARCH NOTE

SCHISTOSOME DERMATITIS IN CHARLESTON COUNTY, SOUTH CAROLINA.

During the summers of 1960 and 1961, snails were collected from several localities with the hope of finding schistosomes. At present two species have been found. One, tentatively identified as *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, was isolated from *Nassarius obsoletus* collected at Fort Sumter in Charleston Harbor. The other, possibly a new species, was isolated from a Marsh Periwinkle, *Littorina irrorata*, collected at Cat Island.

The species from *L. irrorata* produced a severe dermatitis in a volunteer. At this writing the rash is still intense, 13 days after exposure. This is the first report of schistosome dermatitis in South Carolina.

Experiments are underway to infect chicks and parakeets in an attempt to recover adult schistosomes.

(This investigation was made possible by Research Grant G-13307 from the National Science Foundation)—DONALD W. DERY AND PAUL F. TELLER, *Department of Biology, College of Charleston, Charleston, South Carolina.*

DIPETALONEMA WITEI, FILARIAL PARASITE OF THE JIRD, *MERIONES LIBYCUS*. I. MAINTENANCE IN THE LABORATORY

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The filarial parasite *Dipetalonema witei* is found naturally infecting the Libyan jird *Meriones libycus*, a burrow-dwelling rodent living in the open sandy plains of North Africa and Asia Minor. There is some confusion as to the exact taxonomic and nomenclatural position of the parasite.

Chabaud (1952) described the filaria and named it *Dipetalonema blanci*, but later (1957) decided that it was identical with *Litomosa wite* Krepkogorskaya, 1933. The name *blanci* falling into synonymy, Chabaud created a new combination *Dipetalonema vite*, misspelling the specific name which had earlier been emended to *witei* by McIntosh and McIntosh (1935). Yeh (1957), however, divides the genus *Dipetalonema* and resurrects several genera placed in synonymy by Chabaud (1952). Under Yeh's classification, the above mentioned parasite would fall into the genus *Monnigofilaria*. However, we have retained the name *Dipetalonema* as the generic name of this parasite until a settlement has been reached as to its proper taxonomic position.

The parasite is transmitted by the argasid tick *Ornithodorus tartakovskyi*, Olenev 1931, which lives in the jird burrows. A laboratory infection of this parasite was first established by Baltazard et al (1953). Through the courtesy of Dr. Baltazard we obtained infected jirds and ticks in 1953 and since then have successfully maintained the infection in our laboratory.

This paper describes our experience in maintaining the infection and the responses of the parasite to some of the known filaricidal drugs. We have also attempted to compare the usefulness of *D. witei* as a laboratory infection with that of *Litomosoides carinii*, filarial parasite of the cotton rat, which has been maintained on a large scale in this laboratory since 1947 (Hawking and Sewell, 1948).

The Invertebrate Host

The principal vector used in our transmission experiments has been *Ornithodorus tartakovskyi*. This tick breeds readily and can be successfully reared at 29 C and 100 percent relative humidity in small glass tubes containing filter paper and plugged with cotton wool wrapped in bolting silk. Under these conditions the ticks complete their life cycle in 3 to 4 months if given frequent opportunities to feed. Larvae and nymphs were fed on new born rats; adults of the stock culture, on uninfected jirds or cavies. At 29 C and 60 percent relative humidity the ticks remained alive but did not breed.

The ticks were infected by allowing them to feed on the shaved abdomen of a jird anaesthetised with Nembutal and showing 4 or 5 microfilariae per cu mm of tail blood. If the microfilarial count was much in excess of this figure, many of the fed ticks died.

The development from microfilaria to infective larva took 20 to 25 days at 29 C. During the first stage, which covers 11 to 13 days, the larva retains the general appearance of a microfilaria though increasing in size to a maximum of 315 by 12 microns (fig. 5). The second larval stage (fig. 6) is characterized by the persistence of the first larval sheath which projects at both ends. During this stage, which lasts from 6 to 7 days, the gut becomes differentiated and the somatic nuclei are aligned laterally. The larva at this stage measures about 590 by 22 microns. There is rapid development following the second molt which occurs on the 18th or 19th day. The gut is fully differentiated, the caudal extremity develops a pair of lateral projections and there is a great increase in size (fig. 8). The mature infective larva measures 1300 to 1600 microns in length.

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Chabaud (1952) has observed that the early development of the larva appears to take place in the general body cavity until the 14th to 18th days during which period the larva penetrates into the muscles and there continues its development. In this laboratory, larvae have been found in the muscles as early as the 4th day after infection and it would appear that in common with other filarial infections, the whole rather than part of the larval development occurs within the muscles of the vector (fig. 7). The mature 3d-stage larvae leave the muscles and are found distributed freely throughout the host tissue.

The larvae remained alive and infective in the tick for periods up to 203 days at 28 C. The infection rate in *O. tartakovskyi* was almost 100 percent as judged by the presence of infective larvae; there was, however, a considerable wastage of microfilariae as each infected tick contained many dead larvae with arrested development. A single tick may support as many as 50 live infective larvae.

Attempts were made to infect several other

FIGURE 1. Adult jirds (*meriones lybicus*).

FIGURE 2. Adult *Dipetalonema witei* in subcutaneous tissue of jird.

blood-sucking arthropods; these were *Ornithodorus moubata* and *O. savignyi, Triatoma infestans, Cimex lectularius* and *Aedes aegypti.* Development to the infective stage occurred only in *O. moubata;* no development beyond the microfilarial stage took place in the other arthropods. In all these experiments, control feeds with *O. tartakovskyi* showed that the microfilariae were capable of developing to the infective stage.

The particular strain of *O. moubata* used in these experiments was "form A" isolated by Dr. A. Walton at Nyeri, Kenya, which had been maintained in laboratory culture for 6 years. Further transmission experiments showed that *O. moubata* was as efficient a vector as *O. tartakovskyi.* The tick was bred and maintained under the same conditions as *O. tartakovskyi* except that the relative humidity was reduced to 60 to 80 percent.

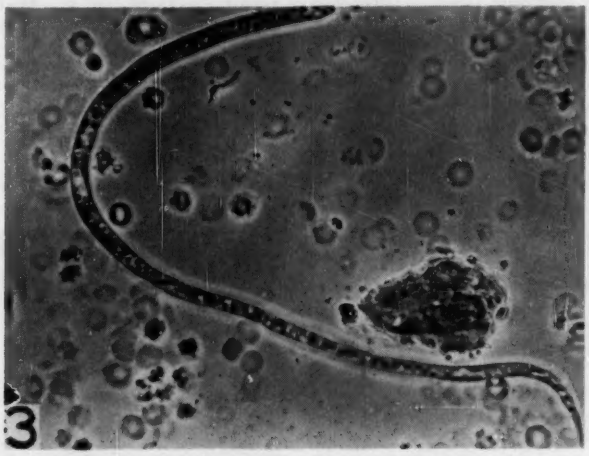

FIGURE 3. Microfilaria of *D. witei* (phase contrast ×470).

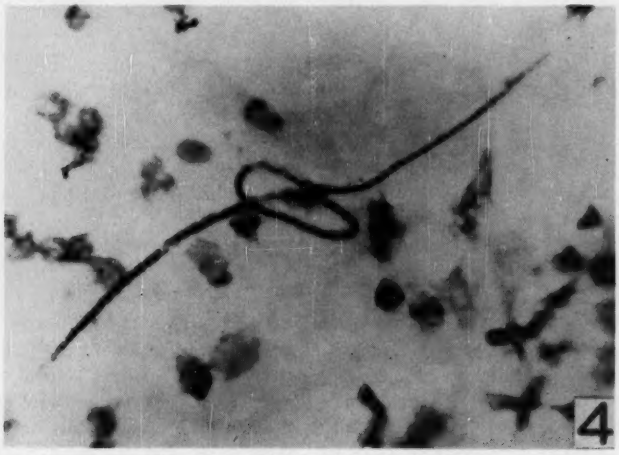

FIGURE 4. Microfilaria of *D. witei* (stained in haemalum ×530).

The Vertebrate Host

The chief vertebrate host used in this work has been the jird, *Meriones libycus* (Rodentia) (fig. 1). Originally three infected jirds were

obtained from Dr. Baltazard and two breeding pairs were received from Dr. Petier in 1953. Since that time a breeding colony has been maintained in the Animal Division of this Institute under the supervision of Mr. L. Gammage, and we are grateful to him for the following information on the care and maintenance of the colony.

Breeding pairs are put together either at weaning or when they are about 3 months old. They are housed in standard rat cages 15 in. by 10 in. by 9 in. with sawdust and wood fibre as bedding. They are fed on stock Institute diet 41 (Bruce and Parkes, 1949) with fresh greens daily and water from licking bottles. Mating occurs readily; the gestation period is 28 days and the average size of the litter is four. The young are born naked and blind but by 15 to 17 days their eyes have opened and they have grown fur. The young are weaned when 21 to 30 days old. A serious handicap to breeding is the high mortality (16 to 30 percent) among the young between birth and weaning; this may be reduced by removing the male when the young are born. The temperature of the animal room is fairly constant at 23 to 24 C; no special precautions are taken to control humidity.

Jirds are easy to handle in the laboratory. They may be held by the base of the tail; they seldom bite. The adults tend, however, to fight among themselves and it is advisable to house them individually in smaller cages (7 in. by 7 in. by 6 in.).

Method of Infection

Most of the jirds were infected by inoculating them with a known number of infective larvae. The larvae were dissected from the tick, suspended in Ringer's solution, and inoculated subcutaneously by means of a syringe and a wide-bore needle. There was no obvious tissue reaction of the host, and this procedure did not appear to damage the larvae unduly. The natural transmission of the infection by feeding infected ticks on clean jirds did not prove satisfactory. Microfilariae appeared in the peripheral circulation at times varying from 42 to 65 days after infection and increased fairly rapidly in numbers to give peak counts about 80 days after infection. Thereafter the counts fell gradually; microfilariae persisted in the blood of some of the jirds for as long as 15 months. The peak counts varied widely from as few as 10 to as many as 800 mf per cu mm. The infection rate in jirds was 84 percent; the number of adult worms recovered post mortem varied from 10 to 100 percent of the number of infective larvae inoculated.

A few jirds were infected by transplanting adult worms taken from other accidentally killed animals. The worms were dissected out in Ringers solution and, after a brief examination were transferred into a subcutaneous pocket in the groin of the new host; the pocket was then sutured. Aseptic precautions were observed in order to reduce the host reaction in the tissues surrounding the worms. Transplantation was successful in all cases when the worms were active and in good condition. Microfilariae appeared in the peripheral circulation of the new host within 2 or 3 days and persisted up to 60 days.

Examination of the Parasites

1. Microfilariae. The microfilariae of *D. witei* are large (175 microns), active, and easily seen in wet blood films with the microscope (×10) (figs. 3, 4). Blood from the tail of the jird normally contains very few microfilariae, but if a general anesthetic such as ether or chloroform is given there is a sharp rise in the microfilarial count within 1 or 2 minutes of the onset of anesthesia. We have found ether to be the safest anesthetic for routine use, but individual animals vary in their resistance to the anesthetic and a few have been accidentally killed. As the depth of anesthesia is not easy to judge, consistent microfilarial counts are difficult to obtain. The microfilarial count is also raised by letting the jird breathe pure oxygen; the microfilariae show no periodicity (Hawking, 1956).

We have investigated the distribution of the microfilariae in several tissues and organs of the jird. A jird was killed by a blow on the head, and organs and tissues were quickly removed and fixed in corrosive acetic fixative. Sections were cut at 5 microns, stained with hematoxylin and eosin, and systematically examined, magnification ×1100. With this magnification it was possible to identify with certainty transverse sections of microfilariae as well as the oblique or longitudinal. Table I summarizes the findings. The microfilarial density was highest in the cardiac muscle and lungs; the skeletal muscle contained less microfilariae per unit volume, but since that tissue constitutes almost half of the total body weight about 22 percent of the

TABLE I. *Distribution of microfilariae* (m. f.) *in the organs of a jird.*

Organ	Vol. organ cc per 100 g	mf per 100 sq mm	mf per 100 cu mm	Total mf in 1000 s	Pct. of total
Blood	6.3	—	100.0	6300	53.5
Lung	0.7	1866	37.3	261	2.3
Cardiac muscle	0.5	3000	60.0	300	2.6
Kidney	0.8	919	18.38	147	2.3
Liver	3.7	351	7.02	259	0.03
Spleen	0.15	153	3.06	4.6	9.3
Skeletal muscle	45.0	487	9.74	4380	37.1
Skin	18.0	9	0.18	32.4	1.4
Brain	1.7	178	3.56	60.5	0.5
(Total)				11,844	100.03

No large blood vessel (100 microns diameter) was included in the areas examined. Microfilariae counted in 100 sq mm were converted into mf per 100 cu mm by the formula of Hawking, Sewell and Thurston (1950); i.e., 1 mf per 100 sq mm = 4.20 a $\frac{3}{2}$ mf. per 100 cu mm.

was given by the fact that the number of microfilariae in the blood of the dead jird was much greater than that in the blood of the living.

Further investigations were made on a second jird. This animal which showed 7 mf per 20 cu mm of peripheral blood was killed by a blow on the head. Immediately after death the blood in the left ventricle of the heart was found to contain 2850 mf per 20 cu mm, whilst that of a peripheral vein contained 77 mf per 20 cu mm. These figures indicate that during the process of dying there was a massive liberation of microfilariae from the lungs, and that these migrated into the left ventricle and parts of the vascular system proximal to it. A similar finding has been recorded by Duke (1960) for

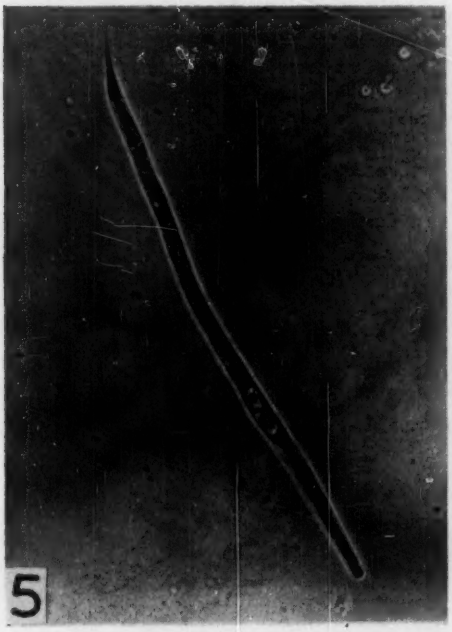

FIGURE 5. First-stage larva of *D. witei* (×520).

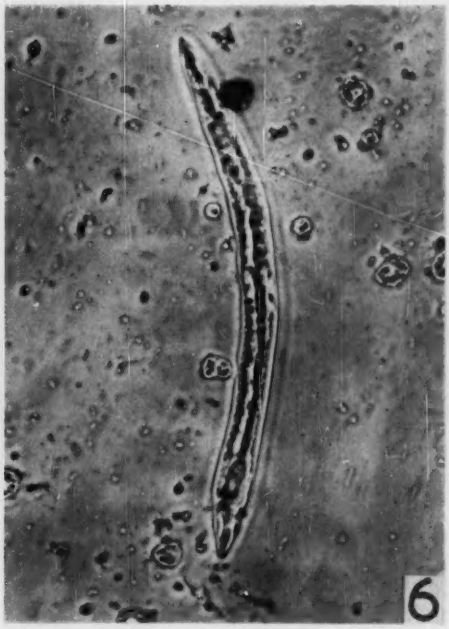

FIGURE 6. Early second-stage larva of *D. witei* (×210).

microfilariae were concentrated there. It is possible that a considerable movement of microfilariae may have taken place during the short time that the animal was dying and before the tissues were removed and fixed. In this case the distribution at post mortem examination would not correctly reflect that in the living animal. Evidence that such a movement had taken place

Loa infection in drills.

It would appear from this work that our estimate of the number of microfilariae in the lung is lower than the number actually occurring in the living jird, and that our estimate of the number in the blood is correspondingly too high.

2. *The adult worms.* The adult worms of

D. witei are found in the subcutaneous tissues and external muscle layers of the jird (fig. 2). The preferred sites appear to be the subcutaneous tissues of the back, axilla, and groin. No worms were found in the belly muscles or adjacent subcutaneous tissues and only five male worms were found in the limbs. The average length of the female worms was 4 cm with a range of 2.9 to 6.9 cm; the males averaged 3.3 cm in length with a range of 2.2 to 4.2 cm.

The recovery of adult *D. witei* at post mortem is not as easy as in some other filarial infections. We have, however, developed a rapid method which enables us to recover the great majority of worms. The dead jird is pinned down with the ventral surface uppermost, and a median incision made in the skin from the mouth to the tail. Lateral incisions are made along the limbs and the skin is peeled back and pinned down. A search is then made for worms on the ventral surface and in the axillae and groins. Cuts are made through the base of the tail and hind limbs, and the animal is then turned forward exposing the muscles of the back and leaving the whole skin still pinned to the dissecting board for examination.

A detailed study of the morphology of the reproductive system, gametogenesis, and devel-

TABLE II. *Action of antifilarial drugs on adult* D. witei *in vivo.*

Drug	Chemical name	M.E.D. cotton rats mg per kg	Dose in jirds mg per kg	Action against worms
Pentostam	Sodium stibogluconate	500–100*	1000	+
K 324	D : (β-carboxy-β-aminoethyl)-4-benzamidethioarsinite	10–20*	20	+
Cyanine 863	1′-ethyl-3 : 6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine	0.1–0.2*	2.0	–
Suramin		100	100	–
BAC 20	Eicosane 1 : 20-bis(4-amino-cinnolinium iodide)	0.5**	5.0	–

* Sewell and Hawking (1950). ** Hawking and Terry (1959).

FIGURE 7. Late second-stage larvae of *D. witei* in muscle of tick (×140).

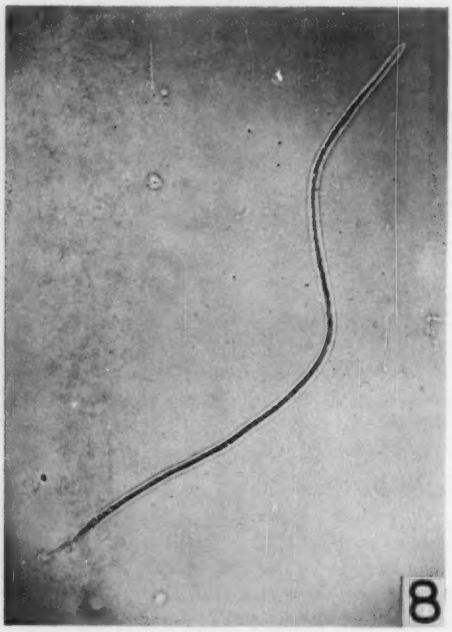

FIGURE 8. Third-stage larva of *D. witei* (×74).

opment of the microfilariae of *D. witei* has been made (Terry, Terry and Worms, 1961).

Alternative Vertebrate Hosts

Several attempts were made to infect other laboratory rodents. Infective larvae were inoculated into hamsters, white mice, laboratory rats, cavies and multimammate rats (*Mastomys coucha*). So far we have been successful in infecting all of 17 hamsters used. Microfilariae have appeared in the peripheral circulation after 50 to 60 days and persisted up to 8 months. The course of these infections was similar to that found in the jirds; at post mortem the adult worms were normal in size and appearance. No microfilariae were seen in the blood of any of the other rodents inoculated with infective larvae and no worms were found at post-mortem. A single attempted transplantation of adult worms from a jird to a laboratory hooded rat was partially successful. Microfilariae appeared in small numbers in the blood 28 days after transplantation but persisted for only a few days. At post-mortem 1 month later the worms were found dead.

Response to Filaricidal Drugs

Several chemical compounds known to be effective against adult worms of other filarial species, notably *L. carinii,* were tested for activity against *D. witei* in vivo. The tests were made with jirds in the early stages of the infection (80 to 100 days); this practice lessened the risk of the results being confused by the natural death of some of the worms.

The tests were based on the method of Sewell and Hawking (1950) for *L. carinii.* Briefly, each jird was given an intraperitoneal injection of the compound under test in solution or suspension daily for 5 days. The dose level of each compound was near to the maximum tolerated dose previously determined in rats or mice. Nine days after the last injection the jird was killed and the worms carefully removed and placed in physiological saline for examination. Those worms which were not moving were placed in an incubator at 37 C for 30 minutes. If again no movement was seen the worms were deemed to have been killed by the drug. A positive action was recorded if all of the female worms were killed.

The results of these tests are summarized in table II. Only those drugs which contained antimony and arsenic were effective in killing the adult worms.

Diethylcarbamazine (Hetrazan, Banocide) was also tested against *D. witei.* The compound has a dramatic action in clearing the microfilariae of *L. carinii* and also *Wucheria bancrofti* from the blood stream. Dosing jirds at a level of 12.5 mg per kg was, however, quite ineffective in reducing the number of circulating *D. witei* microfilariae. The compound showed no activity against the adult worms.

DISCUSSION

D. witei has now been successfully maintained in this laboratory for 7 years. The infection has proved easy to establish and maintain; in this respect our experience has been similar to that of Baltazard et al (1953). The preliminary transmission experiments with hamsters and *O. moubata* suggest that these alternative hosts may be as effective as the natural hosts in maintaining the infection; it is too early to say whether they will prove better hosts.

During the last 5 years we have been able to compare *D. witei* with *Litomosoides carinii* as a laboratory filarial infection. *D. witei* possesses certain advantages over *L. carinii.* These may be summarized as follows:

1. The jird is tamer than the cotton rat and no special skill is needed to handle it in the laboratory.

2. The tick vector is larger, more robust, and lives longer than the mite *Ornithonyssus bacoti* which transmits *L. carinii.* Infective larvae will also survive for longer periods in the tick.

3. The microfilaria of *D. witei* is larger than than that of *L. carinii,* 175 microns compared with 90 microns. It is, therefore, more suitable for demonstration and morphological studies.

The advantages are, however, offset by the following disadvantages:

1. Jirds are more difficult to breed in large numbers than are cotton rats, mainly due to high mortality among the young. The disadvantage may be overcome if the hamster proves a satisfactory alternative host, but it is difficult to obtain repeated blood samples from that animal.

2. The anesthetizing of the jird to obtain microfilariae in the tail blood is time consuming and may be dangerous in unpractised hands. It it also difficult to obtain consistent microfilarial counts because of variation in the depth of

anesthesia.

3. The adult worms are more difficult to recover than are those of *L. carinii* which are confined to the pleural cavities of the cotton rat. This would prove a great disadvantage when large numbers of animals need to be examined, as in chemotherapeutic tests.

4. The resistance of the microfilariae of *D. witei* to the action of diethylcarbamazine casts doubt on the value of the infection from the viewpoint of chemotherapy. If Hewitt et al (1947) had used *D. witei* instead of *L. carinii* as an experimental infection, they would have missed finding the only really effective drug against human filaria.

As a general conclusion, *D. witei* is probably more suitable than *L. carinii* as a small-scale infection in laboratories where the main interests are teaching or morphology. Where large-scale infections are required, as in chemotherapeutic work, *L. carinii* is to be preferred.

SUMMARY

1. The laboratory maintenance of a strain of *Dipetalonema witei*, filarial parasite of the jird, *Meriones lybicus*, and its transmission by the argasid tick, *Ornithodorus tartakovskyi*, are described in detail.
2. The development of the microfilariae to the infective larvae in the invertebrate host was studied. In general, the development is similar to that described for other filariae. A peculiarity is the extreme longevity of the infective larvae which may persist and remain infective in the tick for as long as 203 days.
3. The distribution of microfilariae in the vertebrate host was studied. It conforms in general to that seen in other infections, but the skeletal muscle appears to retain a higher proportion of the microfilariae than is usual.
4. Several known filaricidal drugs have been tested against this infection. Only those compounds containing arsenic and antimony are effective; diethylcarbamazine is not effective against the circulating microfilariae.
5. The infection is compared with that of *Litomosoides carinii* in the cotton rat on the basis of its suitability as a laboratory infection. It is concluded that *D. witei* may be less useful for screening potential filaricidal compounds, but, it is probably easier to maintain on a small scale in the laboratory for carrying out morphological and other studies.

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BIOLOGY OF FILARIOID NEMATODE *DIPETALONEMA SPIROCAUDA* (LEIDY, 1858) FROM THE HEART OF CAPTIVE HARBOR SEALS AND SEA LIONS, TOGETHER WITH PATHOLOGY OF THE HOSTS

ANGELA E. R. TAYLOR,** DAVID H. BROWN,*** DONALD HEYNEMAN,*† AND RANKIN W. MCINTYRE††

The first description of filarial worms from the heart of the harbor seal (*Phoca vitulina*) was given by Leidy (1858) who named the parasite *Filaria spirocauda*. A more adequate description was published later by Lublimov (1927) and, apart from brief comments by Raillet (1899), this was the first description of nematodes from the heart of *P. vitulina* which clearly did not refer to *Dirofilaria immitis*. Anderson (1959) reviewed the taxonomy of these worms and considers that the male described by Lubimov and specimens obtained from the heart of seals caught off the cost of Maine belong to the genus *Dipetalonema* as conceived by Chabaud (1952).

This paper described similar heartworms obtained from a group of five harbor seals (*P. vitulina geronimensis*) held in captivity at Marineland of the Pacific. A group of nine sea lions (*Zalophus californianus*), also exhibited there, were found to be infected with microfilariae; these are described. The adult worms were not obtained for morphological study since the performing sea lions were too valuable to be sacrificed.

MATERIALS AND METHODS

1. *Phoca vitulina geronimensis*

The Harbor, Leopard, or Common seal (*P. vitulina* Linnaeus) is perhaps the most widely distributed member of the true seal family (Phocidae). It is known on both east and west coasts of North America and is also common in British coastal waters and certain areas of the European seaboard. The subspecies *P. vitulina geronimensis* (Allen), being very common in Californian waters, ranges from San Geronimo Island, Baja California, Mexico, to Ventura County, California.

The harbor seal exhibit at Marineland of the Pacific numbered five individuals (details of their capture are given in table I). The seals were fed twice daily on Pacific mackerel, smelt or squid, supplemented with vitamin tablets. Apart from an attack by the louse *Echinophthirius horridus* in November 1958 (see Brown et al, 1960, for details of treatment), these animals were healthy up to January 1959.

TABLE I. *On harbor seals* (Phoca vitulina geronimensis) *captured off the coast of Southern California, for exhibition at Marineland of the Pacific.*

Animal	Age	Weight	Sex	Date	Location
1	16 weeks	40 lbs.	M	Sept. 1954	Paradise Cove, Malibu, Los Angeles Co.
2	3 years	125 lbs.	F	Sept. 1956	China Point, Santa Catalina Island
3	12 weeks	30 lbs.	M	July 1957	Manhattan Beach, Los Angeles Co.
4	12 weeks	30 lbs.	M	June 1958	Malibu Beach, Los Angeles Co.
5	8 weeks	20 lbs.	F	July 1958	Paradise Cove, Malibu, Los Angeles Co.

Seals 2, 3, 4 and 5 commenced to manifest symptoms of disease following that date. These symptoms included: (1) coughing with pale yellow nasal exudation; (2) minor or complete loss of appetite; (3) some alopecia, dermal corrugation of the flanks and several small abscesses (seals 3 and 4); (4) erratic breathing pattern (seal 5). These seals were treated with broad spectrum antibiotics and supplementary vitamins. When antibiotic therapy was discontinued, they relapsed several days later and again showed the same symptoms. Eight weeks after the start of treatment, seal 5 died. The remaining four seals were sacrificed (by euthanisation with intraperitoneal injection of pentobarbitol sodium) and subjected to post-mortem examination (R.W.McI., D.H. and D.B.).

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The right ventricle and pulmonary arteries were found to contain large numbers of adult nematodes (fig. 1), in some instances nearly occluding these structures. The worms were fixed in 70 percent ethyl alcohol plus 5 percent glycerine and cleared in creosote prior to study. A number of specimens were stained in Semicohn's acetic acid stain and mounted in balsam. Thin smears were made from oxalated heart and peripheral blood samples; these were stained with Giemsa or haemotoxylin.

FIGURE 1. *Dipetalonema spirocauda* in the heart of *Phoca vitulina geronimensis*. × 1.

Pathology tests (R.W.McI.). Bacterial cultures were run on specimens of liver, spleen, lung and intestinal tissue from all five seals, using 5 percent bovine agar plates. These were incubated at 37 C. under both aerobic and microaerophilic conditions. Selinite F enrichment and desoxycholate agar cultures were made from intestinal contents.

As seal blood is seldom available for study, differential white cell counts and serological tests were carried out.

2. *Zalophus californianus*

Early in 1960, one of a group of performing sea lions (Mickey) kept at Marineland of the Pacific, exhibited symptoms of disease (lethargy, accompanied by nasal exudate, alopecia, and dermal corrugation with several small abscesses). The similarity of these symptoms to those described earlier for the harbor seals, prompted examination of the blood for microfilariae. Fresh blood samples were obtained (at 11.00 A.M.) by dermal puncture of the rear flippers. The seal offered little resistance to handling provided his trainer (Mr. Arthur D. Thomas) fed him at the same time. The remaining eight sea lions of the exhibit were also examined (at 11.30 A.M.), though it was found to be more convenient to hold these less experienced animals in a restraining cage. Twenty cu mm blood samples were used to prepare thick blood films for microfilarial counts. The samples were spread as thick films, dried, dehemaglobinised, fixed and stained with Giemsa's stain.

More detailed study was made on living microfilariae from heparinised blood from Mickey. The microfilariae were first separated from blood cells (*see* Taylor, 1960, for technique), mounted on agar pads and examined with the phase-contrast microscope. Some were fixed slowly, by penetration of Bouin's fixative through agar, to a coverslip. They were then stained lightly with Giemsa before being examined and photographed by phase-contrast microscopy (Taylor, 1960).

RESULTS

Dipetalonema spirocauda (Leidy, 1858)

The adult worms collected from the heart of harbor seals were found to be *Dipetalonema spirocauda* as described by Anderson (1959). The microfilariae from both the heart and

peripheral blood were unsheathed and exceedingly slender with greatly extenuated caudal extremities. Their length (from stained blood smears) was 235 ± 10 microns and their width 5.4 microns (table V).

Pathology (*R.W.McI.*)

The hematological findings are summarised in tables II, III, and IV.

Seals 4 and 5

Necropsy. The right ventricles of the heart and many of the larger branches of the pulmonary arteries were occluded with adult worms (fig. 1), as were the posterior venae cavae. The right side of the heart showed pronounced dilation and hypertrophy.

Pneumonitis was of a diffuse nature (fig. 2).

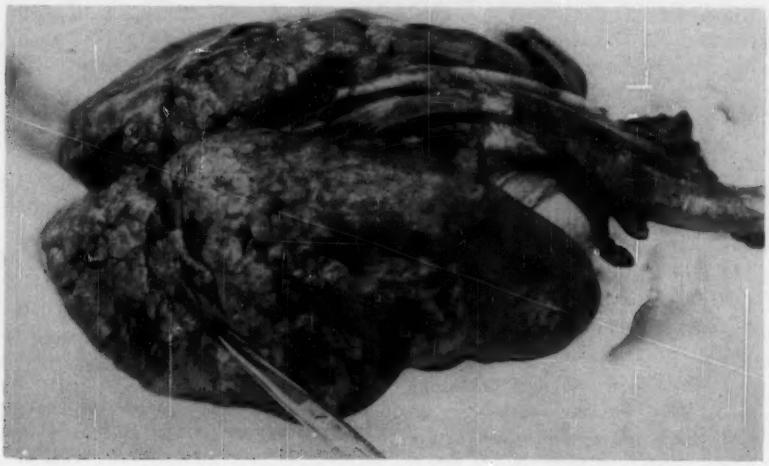

FIGURE 2. Lungs from *Phoca vitulina geronimensis* showing hepatization and pneumonitis. × 0.3.

TABLE II. *Hematocrits of blood from infected* Phoca vitulina.

Seals	Packed red cell volume	Buffy coat	Estimated WBC per cu ml	Estimated RBC
1	52.5	2.5	40,000	12,220,000
2	56.0	2.5	40,000	12,220,000
3	36.0	1.8	24,000	8,000,000
4	37.0	2.0	30,000	8,200,000

TABLE III. *Differential white blood cell counts (in percent) of infected* Phoca vitulina*.

Seals	Monocytes	Lymphocytes	Eosinophils	Basophils	Polymorphoneutrophils	Immature neutrophils
1	1	14	2.0	0.0	49	34
2	1	23	0.0	0.0	69	7
3	2	10	0.0	0.0	44	46
4	1	24	1.0	1.0	49	24

* There was no significant pathology of erythrocytes found. From the white cell count there is a definite leukocytosis and a Schilling's shift to the left. Eosinophilia was not present.

TABLE IV. *Serology of infected* Phoca vitulina.

	Serum dilutions			
	1:25	1:50	1:100	1:200
Brucella abortus (standard plate test)	Neg.	Neg.	Neg.	Neg.
Leptospira pomona	Negative 1:10			
Leptospira-canicola	Negative 1:10			
Leptospira-icterohemorrhagiae	Negative 1:10			

Results of Complement Fixation Tests by the Los Angeles City Health Department:

Influenza Type A	1:8
Influenza Type B	1:8
Psittacosis, lymphogranuloma venereum	1:8
Q fever	1:8

Tests for Weil-Felix and Proteus OX 19 agglutinations were negative.

(Serology for leptospirosis—Rapid plate method U. S. Public Health Services; H. Stoenner technique)

One-third to one-half of the lungs were affected with evidence of congestion and red and grey hepatization. Foci of caseous exudate were scattered throughout the bronchial trees and tracheae, many bronchi being completely obstructed. There was no gross evidence of hydrothorax or pleuritis.

Histopathology. Lungs: There was subintimal proliferation of connective tissue with diffuse thickening of the subintima. Severe endothelial proliferation was evident in many small pulmonary arterioles, with obstruction of the lumina. Many alveoli were filled with an inflammatory exudate composed of leucocytes, lymphocytes, plasma cells, and a few giant cells. This exudate was not confined and was noted throughout many bronchioles. There was loss of normal architecture in scattered areas with extensive interstitial inflammatory changes. Fibroblastic replacement was evident and no viral inclusion bodies or histioparasites were observed.

Liver and kidneys. Extensive cloudy swelling, passive congestion and fatty degeneration of renal tubular epithelium were found. These changes reflected a secondary toxaemia. Microfilariae were not found interstitially, nor were any other significant lesions noted in the tissues examined. Other tissues, including the spleen and lymph nodes, showed some congestion on examination.

Seals 1, 2 and 3

Necropsy. Nematodes were found in the right side of the heart of all three seals; in seal 3 they were also in the pulmonary artery.

The only significant pneumonitis was in seal 3 which showed about 20 percent red and grey hepatization of the lungs with a few caseous occlusions of the bronchi. Congestion of the lungs was apparent in all three animals.

The liver of seal 1 showed diffuse foci of necrosis, approximately 1 mm in diameter.

Histopathology. These findings included inflammatory exudates in the bronchioles. Congestion was a constant finding but no changes in the pulmonary arterioles were seen. No significant lesions were found in seal 1 except for a chronic infection of the liver.

Microbiology. A haemolytic micrococcus species was isolated from abscesses of the lungs of seals 3 and 5. A viridans haemolytic streptococcus was recovered from a lung abscess in seal 3. No enteric pathogens such as *Salmonella* were found.

Many of the histological findings would suggest a septicaemia or toxaemia in three of the seals (3, 4 and 5). Failure to recover organisms from the liver and spleen might be accounted for by the antibiotic therapy. In any event, bacterial etiology should be regarded as a secondary sequel to the heavy parasitism.

One of the curious features observed in the lungs of seals, not typical of many mammals, was the presence of small cartilaginous supporting bands adjacent to the smaller bronchioles. These bands were not circumscribed but seemed to run parallel to the lumina, often four or five bands to each bronchiole. It is suggested that by this histological arrangement a certain elasticity of the air passages would be retained, allowing a marked ability to withstand increased thoracic pressure.

Vector. Large numbers of *E. horridus*, taken from these seals, were dissected (D.H.) in an attempt to find intermediate stages of *D. spirocauda*. None, however, were found.

Zalophus californianus

All the sea lions examined were found to be infected with microfilariae with the exception of one, the counts being as follows: 232, 148, 740, 1460, 184, 432, 384, 0, 6128 (Mickey) microfilariae per 20 cu mm. The microfilariae (length 280 ± 12 microns, maximum width 5.4 microns, *see* table V) are unsheathed and the nuclear column does not reach the end of the tail which tapers to a fine thread. The microfilariae appear similar to those of *D. immitis* as shown in table V, the positions of the excretory vesicle, G cells and the hook-muscle cells being similar in both cases (fig. 3 for phase-contrast photomicrograph of microfilaria from Mickey).

TABLE V. *Dimensions of the microfilariae of* Dipetalonema spirocauda *from harbor seals and microfilariae from sea lion blood, compared with microfilariae of* Dirofilaria immitis (*see* Taylor, 1960).

	Sea lion microfilaria	*Dip. spirocauda*	*D. immitis*
Length	280 ± 12 microns	235 ± 10 microns	260 ± 5 microns
Width	5.4 microns	5.4 microns	4.5 microns
Percentage distance from anterior end			
Nerve ring	22.5	21.7	19.2
Excretory pore	31.2	30.5	29.75
Excretory cell	35.0	37.0	39.5
G_1 cell	57.0	60.0	62.0
G_4 cell	64.0		73.0
Anal pore	68.0		75.0

The periodicity shown by the microfilariae was investigated in one experiment by taking four 20-cu mm samples of blood every 4 hours over a 24-hour period (from Mickey). Figure 4 shows the periodicity of the microfilariae. The

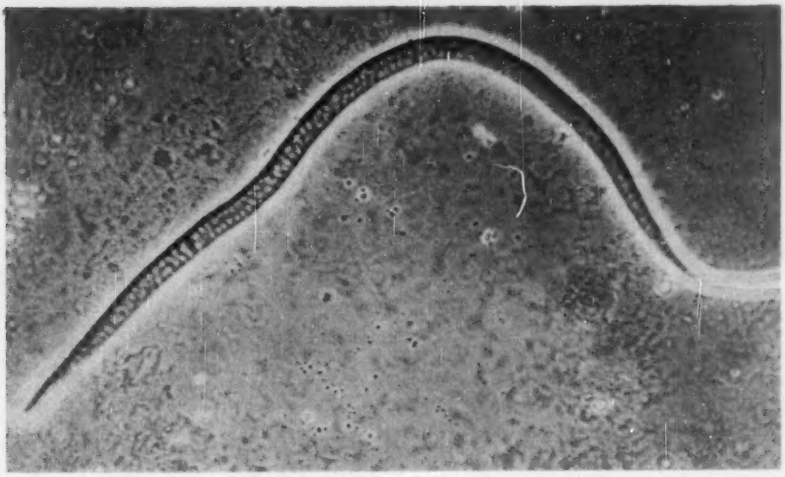

FIGURE 3. Microfilaria from the peripheral blood of *Zalophus californianus*. Stained with Giemsa, phase-contrast photomicrograph. × 125.

maximum number occurred in the peripheral blood around 2.0 P.M. and the minimum number between 6.0 and 10.0 P.M. The low number recorded at 8.0 A.M. may not be accurate as the animal was somewhat fractious at this time and thus the accuracy of these blood films cannot be relied upon.

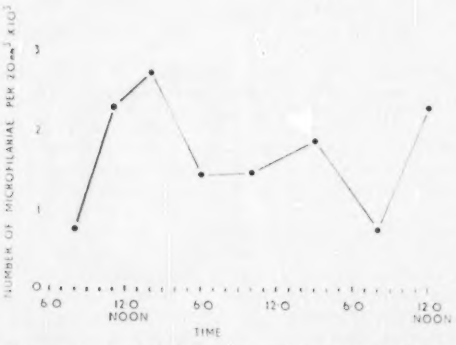

FIGURE 4. Periodicity of microfilariae in *Zalophus californianus*.

In an attempt to determine the vector of this parasite, *Culex pipiens* (mosquitoes) were encouraged to feed on Mickey around noon. Unfortunately, none would feed; it is likely that they were the wrong species of mosquito (they were the only ones available at the time) or, being a laboratory-reared strain, they may have been ill-adapted to feeding on this host.

DISCUSSION

The taxonomy of *Dipetalonema spirocauda* has been greatly complicated by the existence of another heart-worm in seals, namely *Dirofilaria immitis;* however, the specimens described here correspond to the description of *Dipetalonema spirocauda* given by Anderson (1959).

Little is known about the life cycle of these worms. The fact that *E. horridus,* collected from the seals, contained no intermediate stages of filariae indicates that this louse may not be the natural vector, yet it deserves further consideration as it was the only ectoparasite present in large numbers on these seals; however, at Miami Seaquarium, the seals and sea lions are so severely attacked by mosquitoes that they have to be taken indoors each night for protection. Thus mosquitoes might possibly be the natural vector.

The microfilariae taken from the sea lion Mickey might have been *D. immits.* This is suggested both by their morphology and periodicity (Webber and Hawking, 1954). Such evidence is inadequate for final determination, however, and examination of the adult worms would be required.

The sudden onset of clinical symptoms by the seals suggests a recent infection or a reaction from an old one, resulting in pneumonitis. Whichever is the case, early treatment with diethylcarbamazine (hetrazan) might have re-

lieved the symptoms with a possible cure, if the seals could have tolerated large enough doses of the drug (Ziegler, 1950). Therapeutic trials would be necessary to verify this.

SUMMARY

1. Filarioid nematodes from the heart of a captive display of *Phoca vitulina geronimensis* are described and assigned to *Dipetalonema spirocauda* (Leidy, 1858) n. comb. Anderson, 1959.

2. Details of the capture of the seals are recorded. Clinical symptoms of filariasis in the seals, together with pathological findings at necropsy (including serology and blood cell counts) are given.

3. Microfilariae were also recovered from trained sea lions (*Zalophus californianus*), though caged separately from the seals. Periodicity of the microfilariae was investigated, allowing a tentative identification of *Dirofilaria immitis*.

4. Sea lice, *Echinophthirius horridus*, were recovered in large numbers from infected seals, but were found to be negative for developing filariae. That mosquitoes could also be the natural vector is suggested but definitive proof is lacking in either case.

5. Early hetrazan treatment is suggested for captive marine mammals suffering from the symptoms described.

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RESEARCH NOTE

NOTES ON TWO SPECIES OF *TRISTOMELLA* GUIART, 1938 (MONOGENEA: CAPSALIDAE), FROM A SOUTH AFRICAN BLACK MARLIN.

Twenty-one specimens of Monogenea were submitted to H. W. Manter for identification by J. R. Grindley of the South African Museum, Cape Town. The trematodes had been collected from a single black marlin, 60 miles WSW of Cape Recife. They are typical Capsalinae, but 11 have two rows of dorsal marginal spines while the others have only one. The former are *Tristomella pricei* (Hidalgo, 1959) Price, 1960, previously known from the American Pacific; the latter, *T. laevis* (Verrill, 1874) Guiart, 1938, previously known from Long Island Sound

(USA, Atlantic coast), Brazil, and India.

T. pricei is readily identified by its two rows of dorsal marginal spines. The spines of the inner row are slightly larger, more evenly spaced, and number one-sixth to one-fifth the number of spines found in the outer row. Hidalgo's type and 2 paratype specimens from *Makaira mitzukurii* (Jordan and Snyder) at Mazatlan, Mexico, were 12.5 to 13.96 by 12.8 to 13.41 mm. Price (1960, Libro Homenaje al Dr. E. Caballero y C.: 237–244) reported 10 specimens from *Makaira marlina* Jordan and Evermann at Panama Bay which were 8 to 8.5 by 8 mm. Twelve additional specimens from the same collection were available to me, but these measure 3.98 to 10.36 by 3.89 to 11.10 mm. The collector (Sogandares-Bernal) sent another three specimens which I identify as *T. pricei* collected from a sailfish, *Istiophorus greyii* Jordan and Hill (new host record), at Panama Bay. These measure 5.83 to 8.51 by 5.12 to 8.51 mm. The South African specimens are 10 to 11 by 11 to 12 mm. The ventral surface of the body may be almost smooth, may have few papillae (usually concentrated near the mouth and genital pore), or may be conspicuously provided with small papillae over the entire surface. Evidently the papillae are variable structures in this species.

Price (1960) describes the egg of *T. pricei* as "quadrangular, 0.085 by 0.085 mm, with a process 0.020 to 0.045 mm long projecting from each angle." Four of our specimens from Panama (including the smallest one) contain an egg 0.072 to 0.096 by 0.061 to 0.085 mm. On each egg one or more of the four processes is distinctly branched at the tip, and on one egg each process is branched. The posterior process is most likely to be undivided.

According to Royce (1957, U.S. Fish and Wildl. Serv., Fishery Bull. **57** (124): 497–554) the black marlin, *Istiompax marlina* (Jordan and Hill) (syn. *Makaira marlina*), has continuous distribution in warm waters of the Pacific from the Americas to Asia, and has been reported from the Indian Ocean, East Africa, and South Africa. *Makaira audax* (Philippi) (syn. *M. mitzukurii*) has a similar distribution, but is more prevalent in temperate waters. This record from South Africa suggests that *T. pricei* may occur across the Pacific. It has not yet been reported from the Atlantic proper.

The second species has only one row of dorsal marginal spines. Applying Price's (1960) key, the specimens are identified as *T. laevis* rather than *T. poeyi* (Vigueras, 1935) Price, 1960, principally because the diameter of the opisthaptor is one-third or more of the body length. Body size varied from 6.66 to 12.03 by 6.76 to 11.10 mm. Price (1938, Livro Jubilar Prof. L. Travassos: 407–413) differentiated the closely related *T. poeyi* on the basis of (1) posterior haptor smaller in relation to body size, (2) ventral surface of haptor with radiating rows of papillae instead of irregularly arranged papillae, (3) ventral surface smooth instead of with prominent papillae, and (4) vaginal orifice close to common genital pore instead of some distance posteromedian. We have six specimens of *T. poeyi* from Hawaii; two from the "white marlin" which, according to Royce (1957), is the Hawaiian name for *Istiompax marlina* (syn. *Makaira marlina*); four from *Marlina ampla* (Poey) which is the type host in Cuba. Of Price's four points, the most constant and conspicuous difference between the two species is the fact that the vaginal orifice lies only 0.160 mm from the common genital pore in *T. poeyi*, as compared with 0.315 to 0.938 mm away in *T. laevis*. We cannot detect radiating rows of papillae on the opisthaptor of *T. poeyi*, and papillae may or may not be present on the ventral surface of either species.

Although not mentioned in the descriptions of *T. laevis*, Price (1938; and 1939, J. Wash. Acad. Sci. **29**: 63–92) has figured 35 to 44 marginal spines on each side of the body, and states that the spines are 3- to 4-cuspid and 0.038 by 0.020 mm. The South African specimens had a similar number of 3- to 4-cuspid spines (occasionally 2- or 5-cuspid) which are 0.034 to 0.056 by 0.029 to 0.037 mm. In contrast, the spines of our specimens of *T. poeyi* are 60 to 88 per side, 1- to 3-cuspid, and 0.016 to 0.037 by 0.016 to 0.027 mm. The number, shape, and size of the marginal spines may be used to distinguish the two species.

Thus there occur at the southernmost tip of Africa two related species of *Tristomella*, *T. pricei* and *T. laevis*, on the same black marlin. This is a new locality record for each species. *T. pricei* had been reported previously from Mexico and Panama (Pacific Ocean) on *Istiompax marlina* and *Makaira audax*. *T. laevis* had been reported previously from Long Island Sound on *Tetrapturus imperator*, from the "dorodo" of Brazil, and from *Istiompax marlina* (syn. the *"Histiophorus brevirostris"* of Zanzibar and India) at Madras, India. This research was aided by grant G15940 of the National Science Foundation.—MARY HANSON PRITCHARD, *University of Nebraska, Lincoln.*

RELATION OF ECTOPARASITE LOAD TO HOST SIZE AND STANDARD RANGE

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While investigating factors affecting outbreaks of plague, the Indian Plague-Research Commission (1906) found that large rats such as *Rattus norvegicus* in the Bombay area bear roughly twice as many fleas as the smaller species, *R. rattus* (Hirst, 1953). Such information was helpful in making comparative studies of infestations by vector fleas in different parts of the world; a rough factor could be applied to data from one or the other host species to reduce information concerning infestation to a common denominator, then to identify some of the other factors which affect infestation. There were difficulties, however, mainly those associated with the differences in habitats of the two host species. Even in the same building, *R. norvegicus* tends to nest at the lower levels and underground, whereas *R. rattus* tends to nest in the upper drier levels. As a result, the number of fleas and larvae in the nests of the two species survive in different proportions and affect the hosts differently, even though significant interchange of ectoparasites must, perforce, take place in the same buildings.

Milne (1949) subsequently made extensive use of a knowledge of size of host to determine "sheep-equivalent" values of mammals and birds which are alternate hosts of the sheep tick, *Ixodes ricinus,* in England. He observed that the proportion of hosts infested becomes less with decreasing body size and that the animal covering the most ground in contact with the appropriate vegetation will acquire the most ticks. The smaller the animal, the less the breadth of its front and generally the less the linear distance traveled, hence the less the area of ground swept in unit time. At the time Milne wrote, home areas of the animals and birds in question had not been measured except in a very general way.

Since large hosts probably owe their larger infestation to greater area covered as well as to larger size, it is desirable to determine as nearly as possible the influence of each factor. Authors frequently seem to attribute differences in parasitism to physiological, habitat, or behavioristic differences of the host or parasite which actually may be induced by size or amount of activity of the host; many authors use the term "host preference" when referring to differences in infestation.

Three physical factors may affect infestation: the width, or frontage, of a host; the area of its ventral surface, which occasionally touches ground or vegetation; and, for such parasites as lice which may infest most of the body, the amount of body surface available for parasitism. When accounted for, the effect of these rough physical measurements should permit isolation and evaluation of the biological factors.

Since the influence of physical characteristics can be determined best for hosts which are taken at the same time, in essentially the same habitat and when infested by parasites which accept the several species of hosts at random, and since certain chiggers (Trombiculidae) and ticks (Ixodidae) qualify rather well for this type of evaluation, some examples were explored in this study.

RESULTS

Mean Infestation as Related to Host Size and Range

Infestation by chiggers: Some years ago the writer (1956) compared parasitism by chiggers on two species of rats at Sansapor, western New Guinea, and summed up some of the factors that might have accounted for differences in infestation on the two hosts: a small species, *Rattus exulans manoquarius* and a larger one, *Rattus ruber jobiensis* according to Tate, 1951 or, more probably, *R. ruber sansapor,* according to recent examination of my specimens in the U. S. National Museum by Dr. David Johnson. Data from these two species are useful here.

Trombicula deliensis was more numerous on the larger rat; an average of 687 occurred on 17

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in those habitats (rain forest and margin) in which this rat was most heavily infested, whereas an average of only 63 was found on 7 of the smaller rats in those habitats (uncleared beach) in which it was most heavily infested; the larger rat bore about 10 times as many as did the smaller rat. The largest number of chiggers found in the ears of large individuals of each species was about 4,000 and 430 respectively; the larger rat had about 9.3 times as many as the smaller.

Unfortunately too few of the two host species were collected in a single habitat for satisfactory direct comparison. Nevertheless, 11 of the large rats from uncleared native gardens had an average of 223 chiggers per infested rat, whereas 26 of the small species taken at the same time and place had an average of only 5.4 per infested individual. Neither had acquired nearly so many chiggers as it could physically accommodate, but the larger rat had 41 times as many chiggers as the smaller.

The smaller rat averaged about 33 mm across at the midriff, and the larger about 58; it was 1.8 times as large. If other factors did not influence the amount of infestation and if "frontage" only were operational, one might expect only 1.8 times as many of this chigger on the large as on the small rat. Obviously 1.8 is not sufficient to account for the large difference in infestation (9.3 to 41 times); frontage, at least in this case, is an inadequate explanation.

The length of head and body of adult representatives of the smaller rat from Sansapor measured 114 mm, and that of the larger rat measured from 230 to 252 mm. A rough approximation of the ventral area can be had by multiplying length by width. The areas are about 38 sq. cm. for the smaller rat and between 123 and 146 sq. cm. for the larger rat, or about 3.5 times as large. Even the least difference (10) in infestation reported above for the two rats is not completely accounted for by this difference in physical measurement (3.5). The place, amount, kind, or time of activity of the host probably are influencing factors. Adult male mammals of many species are known to travel more extensively than females. Worth (1951) noted that the greater numbers of ectoparasites found on male cotton rats, *Sigmodon hispidus*, and more frequent parasitism suggest a measurable relation. Tanaka and associates (1958) also reported that the rate of parasitism seems to depend on range. Those species with large home ranges should accordingly acquire large numbers of those parasites which lie in wait for a host. Some data gathered at Sansapor, western New Guinea, plus a number of observations by others are available for making comparisons.

Eight *R. ruber* which were caught in live traps by the writer, marked (by clipping of the toes), released and re-observed 4 or more times each from October 16 through December 26, 1945, had a home range of 3.6 acres as measured by Hayne's method (1949). The cage traps were set initially in grids over a 160-acre area, 110 yards apart, were left in position 4 nights (and days), then moved one-third of the way north (18.3 yards) into intervening spaces; followed by a similar move westward. The last observation was one made by using 160 rat snaptraps in the spaces not already trapped, northward and westward of the stations last subjected to live-trapping.

I did not obtain similar data for more than three *R. exulans*, having made most of my observations in the habitat where this species was uncommon. However, observations by others on home range can be used since it is a characteristic of each species and, though subject to fluctuation with time and other conditions (Mohr, 1947), is not unreasonably changeable in equivalent habitats. In 45 records of recapture of four *R. exulans* that had been marked and released on Guam by Baker (1946) 22 percent of the catches were made in the initial place captured, 53 percent at stations that were from 5 to 25 yards apart and 25 percent at stations that were more than 25 yards apart. When areas of activity are calculated from Baker's data by Hayne's method, 68 percent of the points at which the rats were recaught are found to lie within 19.3 yards of center; the diameter of this calculated range is 38.6 yards. (Harrison, 1958, refers to the diameter of the area in which 68 percent of activity occurs as "standard diameter.") These terms, "standard radius" and "standard home range" (which can be calculated from standard radius) will be used further in this paper.

In Hawaii, Spencer and Davis (1950) reported approximately 68 percent of 49 *R. hawaiiensis* (= *exulans*) recaptured within 33 yards of center. The standard diameter is 66 yards. In Mayala, Harrison (1958) reported 68 percent of 36 *R. exulans* captured within 39

meters (42 yards) of center. The standard radius of all varied from 19.3 yards to 42 yards and the mean standard home range was 0.8 acres. The standard observed range of *R. ruber* is 4.5 times that of *R. exulans*.

Since the larger rat has a ventral surface 3.5 times that of the smaller one, and a home range 4.5 times as large, it should tend to bear about 16 times as many chiggers when taken at the same time and place. As noted above it bore 41 times as many, which leaves a difference of 15. This considerable difference, which cannot be accounted for on the basis of size of the rats and standard home range, may be the result of purely physiologic, microhabitat, or behavioristic differences in these rodents. There is strong evidence that this is the case; *R. exulans* is reported to be active during daytime as well as at night (Marshall, 1957), and *T. deliensis* and *T. akamushi* to be active after nightfall and until the dew goes off about mid-morning (Philip, Traub and Smadel, 1949). *R. exulans* does considerable climbing in low vines, shrubs, and coconut trees, although it nests on or close to the ground (Marshall, 1957). I found no indication that *R. ruber* might be a climber at Sansapor. The residual difference in infestation might also be caused by behavior of the ectoparasites. *T. akamushi,* for example, seems to occur most frequently and in greater numbers on birds than on mammals, even in the same habitat. This seeming ability to "choose" a host, or some adaptation which might cause it to avoid mammals either directly or indirectly until a certain physiologic condition, say a certain amount of starvation, were attained after a few days, could bring about considerable selection. Whether this is the case has, of course, to be determined by future investigators.

Although no house mice, *Mus musculus,* were taken in the Sansapor area, their theoretical position should be considered here. Traub and associates (1954) note that in Korea, the house mouse "was the second most abundant mammal collected" in areas where a study was being made of ectoparasites but "very seldom carried ectoparasites," whereas the striped field mouse, *Apodemus agrarius manchuricus,* bore an average of from 1 chigger in July to 80 in October.

In a study of the home range of house mice out-of-doors on Guam, Baker showed that, of 32 mice recaptured 169 times, over 68 percent were taken within approximately 13 yards of center; the standard home range was no greater than 0.13 acre. Since the ventral surface of the house mouse (about 2.7 sq cm) is about 71 percent that of *exulans* and since its range is about 16 percent, it may be expected on this basis to bear only 11 percent as many ectoparasites. Presumably then, if house mice had been present in the native gardens surveyed at Sansapor, the infested mice would have borne only about 3.5 *T. deliensis* since *exulans* bore 32 (Mohr, 1956). And since only 57 percent of the *exulans* were uninfested, fewer of the mice would have been infested. Percentages will be discussed further on.

Comparative data also are available for standard diameter (Harrison, 1958) and infestations by *T. deliensis* and *T. akamushi* on *R. exulans, R. whiteheadi* and *R. argentiventer* (Philip, Traub and Smadel, 1949; Harrison, 1956); for *R. jalorensis* (Harrison, 1956); for *R. rattus argentiventer* (Traub and Frick, 1950); and for mixed populations of *Rattus rattus* and *R. mulleri* (Traub et al, 1954). Though the data on chigger load were not taken at the same time and place as the data concerning the activity of the rats, they are nevertheless revealing.

R. whiteheadi, a small, forest-inhabiting rat which attains a length of 94 mm and a width of about 27 mm, has an exceptionally large standard home range for a small rat (3.6 acres) so far as observation of 10 individuals shows. These bore an average of 64 *T. akamushi* and *T. deliensis* (Philip, Traub, and Smadel, 1949). *R. exulans,* which is slightly larger, had a standard range of 1.0 acre and an infestation averaging 144 chiggers (Philip, Traub and Smadel, 1949; *R. exulans* lives in grassland or grassland and scrub. If the chiggers had been as numerous in forest where *whiteheadi* lived as in nearby grassland where *exulans* lived, one would expect *whiteheadi* to have acquired 3.6 times as many as a result of its wide range. But since its ventral surface is only 0.7 times as large it may gather only 2.5 times as many. Actually it acquired 2.3 times as many during the same trapping period and in the same general area.

In the case of these species, comparisons are not quite so ideal as in the case of the two New Guinea species because they were from different habitats which, though investigated together and were only yards apart, quite possibly had different degrees of infestation. Nevertheless,

the two factors seem to account for the difference in infestation.

Infestations by chiggers on *R. argentiventer* varied between 48 and 550 and the mean, based on 165 rats, was 222 (Philip, Traub and Smadel, 1949). Its observed standard range, calculated from Harrison's standard diameter, was 1.2 acres, and it bore about six times as many *T. akamushi* and *T. deliensis* as did *R. exulans* from the same area. Since it is larger with larger range most of the difference seems to be accounted for.

In another habitat of 10 acres of grassland and scrub where the rats were taken together, *R. whiteheadi* consistently bore the lighter load of chiggers (a mean of 0.6); *R. exulans* was next with 1.0; *R. jalorensis* third with 15, and *R. argentiventer* fourth with 78 (Harrison, 1956, p. 113). The standard diameters of home ranges of these rats were, respectively, 136, 78, 102, and 80 (Harrison, 1958). Although the observed ranges were similar for *exulans* and *argentiventer,* the former bore only 28 percent as many chiggers. Since the ventral surface of *exulans* is 29 percent that of *argentiventer,* much of the difference in infestation may be the result of size. And, since *R. jalorensis,* about as large as *R. argentiventer,* bears only 19 percent as many chiggers and has a range 1.4 times as large, its structure, behavior, or selection of habitat must account for any difference that cannot be attributed to experimental error. This rat does inhabit mixed scrub and grassland, whereas *argentiventer* inhabits more purely grassland, though there is some overlapping of home ranges. *R. jalorensis* also climbs more (Harrison, 1957); much of the difference in infestation is attributable to bionomics.

The number of chiggers on a mixed lot of *R. mulleri* and *R. rattus* (which bears no more chiggers in a given habitat than *R. argentiventer*) varied from 220 through 1200 (Traub et al, 1954) and the mean based on 143 rats was 669. The unknown number of *R. mulleri* in the sample brought the average number of chiggers per rat nearly to the level (687) reported by Mohr (1956) for *R. ruber sansapor* in spite of the handicap by an unknown number of *R. rattus* in the sample.

The standard diameter of *R. mulleri* is 117 meters (Harrison, 1958), and its standard home range is 2.7 acres which is 75 percent of that (3.6 acres) of *R. ruber.* It is about as large as *R. norvegicus,* head and body measuring about 9 inches long (180 mm) (Tate, 1947), and about 71 to 78 percent as large as *ruber.* It is probably, therefore, that *R. mulleri* is infested heavily for approximately the same reasons that apply to *R. ruber.*

Infestation by ticks: Data of Green, Evans, and Larson (1943) are useful in connection with study of relation of ventral area to acquisition of a tick, *Haemaphysalis leporis-palustris,* which, like the chiggers, lies in wait for hosts on the ground or in low vegetation. Cottontail rabbits (*Sylvilagus floridanus*) and snowshoe hares (*Lepus americanus*) were studied in the same general area at the same time. Both species, and ground-inhabiting birds, are hosts to this tick.

From September 20 through October 15, 1938, 8 cottontail rabbits and 19 snowshoe hares were collected by shooting or cage-trapping. The rabbits bore an average of 425 ticks (with a range of from 20 to 1,166) and the hares an average of 1,536 ticks (with a range up to 4,739). Whether one bases his conclusion on the average infestation or on that of the animal in each group with the largest number of ticks, it is apparent that each hare carried about four times as many ticks as each rabbit. Green, Larson and Evans speculate that a difference in choice of habitat may account for the difference in infestation.

The hares average about 443 mm long and 100 mm wide; the rabbits, about 399 mm long and 80 mm wide. Their ventral surfaces approximate 443 sq cm and 319 sq cm, respectively. The rabbit has a ventral surface only about 72 percent of that of the hare; on this basis, the rabbit should bear an average of 1,075 ticks. This leaves a difference of 650 to be accounted for by other factors.

Comparative studies of the size of home range of hares and rabbits are scattered and not made in such a way as to be easily adapted for comparison. Nevertheless, four or five acres are consistently mentioned for the rabbits and 10 for the hares, though areas of greater size are mentioned. Further, in Missouri, Schwartz (1941) reported that the observed range of the cottontail was 1.4 acres for males and 1.2 for females and that the data from other authors as 8.3 acres and 3.0 acres also in Missouri, and 3.6 and 2.2 acres in Michigan, and from 0.11 to 0.58 acres in Iowa. Adams (1959) indicated 7.7

average for 68 percent of 298 observations of adult snowshoe hares observed by him.

If the area used by the rabbit is half that used by the hare, one might expect an infestation on this basis of 0.5 that of the hare; the combined factors of ventral surface and standard range indicate that the rabbits' infestation should, on these bases, be about 537 ticks. Although obviously greater refinement of data is needed for better analysis, it is probable that the rabbit actually did select subhabitats that were not so suitable for the tick as were those of the hare, or that other biological factors were at work. However, not all the difference in infestation is to be sought in differences in habitat.

Percentage of Hosts Infested

T. deliensis in the Sansapor area failed to infest a percentage of both *R. exulans* and *R. ruber*, especially in the habitats in which the number of *deliensis* per infested rat was low.

Observers have noted (Philip, 1948, p. 185) that a peculiarity of tsutsugamushi disease (transmitted to humans by *T. akamushi* and *T. deliensis*) which was re-emphasized all during World War II was the observed localization of infection within larger endemic areas; adjoining army units established under practically identical conditions with relation to jungle margins in the same kunai, lalang or cheena fields or coconut groves and scrub often showed "spottiness" in incidence. In one example, five cases occurred in one half of a split squad, the other half of which remained free of infection though it was deployed approximately 200 to 300 yards away. "It is probable that such localization is a reflection of restricted movement within the local environment of mite-bearing rodents, permitting much slower spread of the infection than might at first be thought" (Philip, 1948).

Since chiggers travel little under their own power, since eggs of each chigger are laid over a very small area, and since only a portion of the standard range of any rat is likely to support chiggers, spottiness should be revealed in the percentages of the various rat species infested.

About 5 days is the usual maximum time required for *T. deliensis* to engorge and desert the host, and an unknown number of additional days ensue during which those chiggers that die on the host remain attached. However, if one goes by the presence of living chiggers, a period of more than 5 days must elapse before any rat, once infested, will lose all of its living chiggers.

The range of any host must, therefore, be almost completely free of chiggers ready to attach for 6 or more days if the host uses all of its range in such a way as to become infested, or half the range must be free if the host spends all of its time for 6 or more days in the free half. Probably the rat uses most of its standard range within 3 or 4 days so that, if chiggers in it are ready to attach, the rat will be continuously free or continuously infested if its range is infested by more than a few widely scattered chiggers.

If the standard range of *R. ruber* is somewhere between 2.7 and 4.4 acres, say 3.6 acres, in the moist native garden and uncleared rain forest and margin where 100 percent of the rats are infested (Mohr, 1947), one can visualize the areas as 3.6-acre circles or hexagons of which 100 percent have chiggers. In stands of short mixed vegetation and dry native garden where 16 of 19 *ruber* were infested, there must have been some 3- or 4-acre uninfested units (unless, of course, some of the sample of rats had drifted into the area from some uninfested habitat where they were resident, an improbable event). But only 57 percent (of 21) *exulans* were infested in dry native gardens. Since three *ruber* in this area were uninfested and since 3×3.6 (acres) equals 11, then at least 11 *exulans* could very well have resided in the areas where the *ruber* were free of chiggers. However, trapping revealed more complete interspersion; at least 16 of the *exulans* were taken within *ruber* ranges. Since these *exulans* were interspersed among the populations of *ruber* and since their standard range is about 1 acre, rather large sections, say about a third, of the *ruber* ranges either were without infestations by chiggers ready to attach, or the negating action of *exulans*, such as being most active during the chigger-free portion of the day, was effective. The behavior of *exulans* which might keep it relatively free of chiggers has already been noted.

Here we may speculate what might have been the percentage of house mice infested, based on the relative size of their known (to date) standard range (0.13 acre) out-of-doors. About 6.3 percent would have been infested. Other writers comment on the low percentage

of house mice infested by fleas and mites (Eskey, 1934, Cole and Koepke, 1947).

Studies made by Elton and Keay (1936) provide further percentage data concerning infestation, in this case of the Eurasian wood mouse, *Apodemus sylvaticus*, and bank vole, *Clethrionomys glareolus*, by *Trombicula autumnalis* in woodlots near Oxford, England. They also got data from the field vole, *Microtus agrestis*, but since this host was taken mainly in grassland, the difference in infestation between it and the other two may be the result of a different chigger population. Elton and Keay reported only on percentages of the mice infested by *T. autumnalis*, not on the mean number of chiggers per rat.

Month by month, beginning in January, the following percentages of bank voles were infested: 100, 100, 100, 49, 8.7, 0, 42, 83, 91, 83, 66, 47 based on the following numbers of voles examined, respectively: 27, 25, 34, 43, 23, 31, 26, 35, 61, 40, 50, 38. No woods mice were observed to be infested from January through June although 120, 77, 75, 77, 21, and 34 mice were examined during those months. Beginning in July, the following percentages of woods mice were infested: 6.5, 28, 52, 41, 9.0 and 1.5. It is convenient here to use a single comparative semi-annual percentage, a mean based on the sum of the percentages for 6-month periods, divided by 6.

It is surprising to this writer that two species of hosts in the same woods would have such different infestations during the first 5 months (72 and 0 percent, respectively). One is led to suspect that perhaps two different species of *Trombicula* were involved. Nevertheless the mean percentages for the last 6-month period are 68 and 23, respectively. They seem fairly to represent *T. autumnalis*.

The average home range of male wood mice captured 3 or more times is 0.72 acres; of females, 0.24 acres (Miller, 1958). Brown (1956) indicates that 73 percent of both bank voles and wood mice moved less than 30 yards in a 24-hour period, and 92 percent less than 60 yards (Brown, 1956). According to him, the average home range of male bank voles is 0.44 acre; and that of the male wood mouse, 0.64 acre, or 1.4 times as great. On the basis of difference of range, one would expect more *T. autumnalis* on the wood mouse. But the wood mouse is only about 4 inches long (Tate, 1947) and the bank voles from about 4.5 inches, and their ventral surfaces approximately are 6.0 and 7.7 square inches. One would expect a slightly greater infestation (say 1.3 times as great) on the bank vole on this size basis. This offsets the favorable difference in range for the wood mouse. The difference in infestation therefore indicates the extent to which other factors than ventral surface and standard range favor the acquisition of *T. autumnalis* by the bank vole. It is possible that the bank voles' selection of microhabitat is the ruling favorable factor but, with these physical features accounted for, isolation of the more purely biologic features should be more effective.

Jameson and Brennan (1957) also record percentages of hosts infested by two species of chigger in the same habitat. *Euschongastia radfordi* and *E. peromysci* were rather common on four species of mice (*Peromyscus maniculatus, P. boylii, Clethrionomys occidentalis,* and *Microtus longicaudus*) and "no host preference was discernible." However, *E. radfordi* was much more abundant on mice inhabiting brushfields and *E. peromysci* was more prevalent on mice living in coniferous forests, based on observations of 448 *P. maniculatus* and 796 *P. boylii* from brushfields.

Since difference in percentage of *P. boylii* (86) and *P. maniculatus* (76) infested by *E. radfordi* are significant, it will be informative to examine their relation to the sizes of the two hosts. *P. boylii* is 3.75 to 4.50 inches long and *P. maniculatus* is 74 to 88 mm long; *P. boylii* is the larger, hence would be expected to gather more chiggers if they were rather uniformly distributed in the habitat unless some peculiar habit caused *P. maniculatus* to contact this chigger more often, or unless it was more often selected by this chigger. *E. peromysci*, on the other hand, occurred on a greater percentage of the *P. maniculatus* (about 40 vs. 23) in brushland.

Since this difference occurs, and since this chigger is more abundant in forest, it seems probable that *P. maniculatus* must spend more time in situations which are tolerable to this chigger, or that it otherwise comes into greater contact with the chigger.

CONCLUSIONS AND SUMMARY

A review was made of the relation of infestation by certain chiggers and ticks to the size

and standard home ranges of pairs of several host species taken together so far as possible in the same habitats. The hosts of the chiggers vary in size from house mice, *Mus musculus*, with a ventral surface of about 2.7 sq cm, and standard home range out-of-doors of 0.13 acre, to New Guinea rats with a ventral surface of about 146 sq cm and standard home range of 3.6 acres. The hosts of the bird and rabbit tick vary in size from that of the cottontail rabbit, *Sylvilagus floridanus*, with a ventral surface of about 319 sq cm to that of the snowshoe hare of about 443 sq cm.

What generally passes for "host preference" among certain parasites is suggested to be partly the result of: (1) a difference in amount of area of the host available for infestation, or exposure to infestation; (2) a difference in amount of area covered per individual, expressed as size of standard home range as defined by Hayne (1949) and Harrison (1958). The remaining differences in infestation of the several hosts are, then, a measure of more purely biological factors such as difference in devotion of time to sub-habitats in a given habitat such as bushes or trees, unshaded areas as compared with shaded areas, or to physiologic condition, cleansing ability, intensity of activity, and observational error.

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AN EPISODE OF PSEUDO-PEDICULOSIS

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During the spring of 1958, several thousand children were sent home from public schools in and adjacent to Portland, Oregon, because of suspected pediculosis and a similar condition was found in the adult population. Diagnosis was based on the presence of numerous objects that resembled nits, i.e., the eggs of *Pediculus humanus capitis* DeGeer, attached to the scalp hairs. Local health authorities disagreed on the identification of these objects and the failure to find nymphs or adult lice on the majority of people examined was further cause for doubting the diagnosis of pediculosis. Intensive search did reveal a few instances of real louse infestation. Numerous specimens, including dandruff, lint and dirt, were submitted for examination. The "epidemic," either imaginary or real, spread from the suburbs of Portland to the City of Portland schools and additional children were sent home to be deloused.

A study showed that many individuals, both children and adults, did have small whitish objects firmly attached to the hairs. These were rather consistent in size and shape and were not just accidental flecks of dandruff or dirt. If these were prevalent previously in Portland or present in the population elsewhere, they had escaped medical attention insofar as we have been able to determine.

A number of these "nits" from a few individuals were examined microscopically (figs. 1, 2, 3, and 5). It was readily established that they were not true nits, i.e., eggs of *P. h. capitis* (fig. 6), and health officials were so informed. When moistened with dilute sodium hydroxide, the bodies disintegrated into an amorphous mass of cellular debris and left the hair shaft undamaged. This eliminated the possible diagnosis of "piedra," a fungus infection of the hair. The disintegrated mass contained many epidermal cells, obviously from the scalp. Out of about 20 nit-like objects examined, 3 contained a single mite which we have identified as *Demodex* sp., probably the common follicle mite of man, *Demodex folliculorum* (Simon)

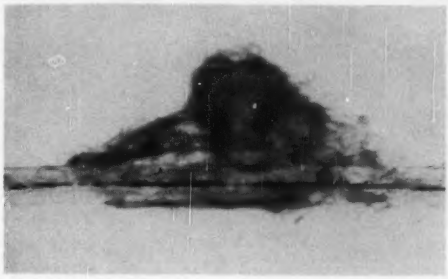

Figure 1. Nit-like objects on human hair. ×56.

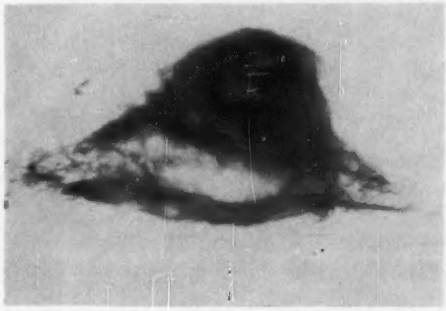

Figure 2. Nit-like objects on human hair. ×56.

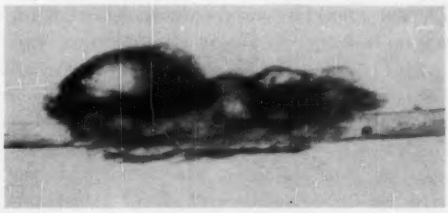

Figure 3. Nit-like objects on human hair. ×56.

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(figs. 4A and B). Dissection under the compound microscope was necessary to observe this parasite in the cellular debris.

Figure 4A. × 100.

Figure 4B. × 330.
Demodex from nit-like object

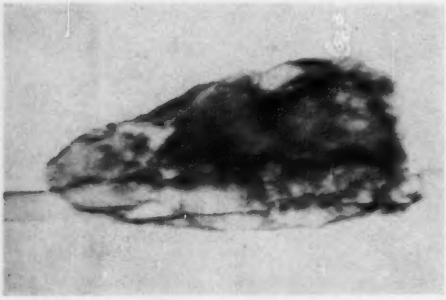

Figure 5. Nit-like objects on human hair. × 60.

D. folliculorum is a common parasite in the sebaceous glands of the face in man. It usually does not cause any inconvenience, but at times has been reported as causing irritation and lesions, even in the scalp. We have found no record of its occurrence in objects attached to the hair. These pseudo-nits are not eggs of the mite since they are many times larger than the mite. The only plausible explanation we can give is that the scalps were infested with *Demodex* which possibly caused some irritation in the hair follicles. The secretion and pus cells in the hair follicle entrapped some of these mites and adhered at the same time to the hair shaft. As the hair grew out it carried this small pustule and its entrapped mite which in time came to be several inches from the scalp. The presence of epithelial cells plus follicular mites is ample proof that these bodies were formed in the hair follicles and were not of extraneous origin. They were quite conspicuous in dark hair. They resembled nits closely enough to pass as such on casual observation (fig. 5) and were identified as such by professional health workers.

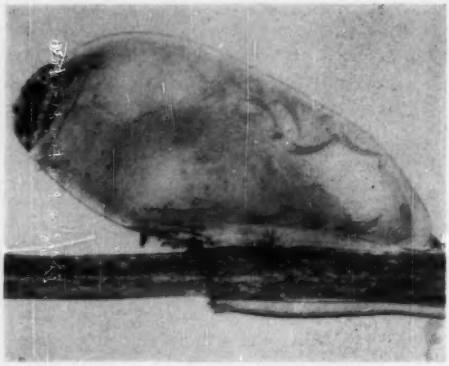

Figure 6. True nit; egg of *P. humanus* on human hair. Tarsal claws on embryo visible within. × 60. (All photographs by N. J. Kramis, Rocky Mountain Laboratory)

We are still far from certain that *Demodex* was responsible for the objects in the hair. The presence of *Demodex* in these objects may have been entirely accidental. So far we have not found any record in parasitological literature of a comparable episode.

COMPARATIVE INTERNAL MORPHOLOGY OF SUBGENERA OF *ARGAS* TICKS (IXODOIDEA, ARGASIDAE). I. SUBGENUS *CARIOS: ARGAS VESPERTILIONIS* (LATREILLE, 1802)

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The present report is the first of a comparative series on internal anatomy of species representative of *Argas* subgenera occurring in Egypt. These studies are designed to correlate internal and external morphological characteristics and to test contemporary subgeneric concepts on the basis of internal structural criteria. Differences in external morphology of various groups in this genus and species with structural characteristics approaching those of the genus *Ornithodoros* have resulted in conflicting views of subgeneric divisions within the Family Argasidae, some of which may be resolved in part by comparative studies such as the present one. The genus *Argas* was reviewed by Nuttall et al (1908), and its species and subgenera are now being revised by Hoogstraal.

In the Cairo area, Hoogstraal (1952) found two bird-parasitizing species, *Argas persicus* and *A. reflexus,* and three bat-parasitizing species, *A. boueti, A. vespertilionis,* and *A. transgariepinus. A. confusus* was later discovered in Egypt (Hoogstraal, 1955) and the subgenus *Chiropterargas* was erected to include *A. confusus* and *A. boueti.* Hoogstraal (1956) and Hoogstraal and Kaiser (1957) reported *A. (Ogadenus) brumpti,* an African parasite of reptiles and mammals, in the southeastern corner of Egypt near Luxor. Next, Hoogstraal (1957) redescribed the poorly known *A. transgariepinus,* erected the subgenus *Secretargas* for it, and compared it with the subgenus *Argas,* which he redefined. *A. vespertilionis* was then studied in detail and the subgenus *Carios* redefined (Hoogstraal, 1958). Hoogstraal and Kaiser (1958, p. 85) reported that the common pigeon parasite of Egypt could be related to the name *A. hermanni.* Hoogstraal and Kohls (1960A) redefined the European *A. r. reflexus* and (1960B) redescribed the African form as *A. reflexus hermanni.*

The five subgenera of *Argas* present in Egypt are *Argas* Latreille, 1796, *Carios* Latreille, 1796, *Chiropterargas* Hoogstraal, 1955, *Secretargas* Hoogstraal, 1957, and *Ogadenus* Pospelova-Shtrom, 1946. Of these, the internal anatomy of only the subgenus *Argas* has been studied. Heller (1858), Patton and Cragg (1913), and Robinson and Davidson (1913A, B, 1914) described the internal structures of *A. persicus* and Pagenstecher (1861) those of *A.r.reflexus.* As will be elucidated in the final summarization of the present series of papers, the internal systems that show variation in subgenera of *Argas* are the digestive, reproductive, and respiratory. These systems in the subgenus *Carios,* represented by *A. vespertilionis,* are described below.

MATERIALS AND METHODS

Specimens used in this investigation were adults of *A. vespertilionis,* collected from bat-infested caves in the Abu Rawash area near Cairo. They were kept alive at 29° C and 40 percent R.H. At least 50, unfed and fed, specimens of both sex were examined.

For dissection, live ticks were dissected in saline or in 50 percent alcohol. Occasionally, dissected specimens were fixed with Formol-saline or Carnoy's fluid to make certain organs more clearly visible during examination.

OBSERVATIONS

Digestive System

The digestive system consists of the alimentary canal and its associated salivary glands. According to Robinson and Davidson (1913B) the alimentary canal is divided into: fore-gut which includes the buccal cavity, pharynx and oesophagus, mid-gut including stomach with its branches, rectum and rectal sac, and hind-gut, represented by the anal canal and ending at the

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anus.

The capitulum of *A. vespertilionis* has been described by Hoogstraal (1958). The capitulum lies in fairly deep camerostome closely bounded by coxa I, from which it is separated laterally and posteriorly by a "fleshy ridge." The basis capituli is short and lies basally. Within the basis capituli lie the first two portions of the alimentary canal; i.e. the buccal cavity and pharynx.

Buccal cavity. The buccal cavity is a small, triangular sac, similar to that of *A. persicus* described by Robinson and Davidson (1913B). According to these authors, the buccal canal, lying between the chelicerae and hypostome, terminates posterior of the base of the hypostome and leads to the buccal cavity. The roof and floor of this cavity are formed by the backward and direct continuation of the cheliceral sheaths, and the portion of the hypostome forming the floor of the buccal canal and hypostomal gutter respectively. The pharynx opens into the floor of the buccal cavity and the salivary ducts open at its posterolateral ends.

Salivary glands. The paired salivary glands lies ventrally in the anterolateral region of the body cavity. It extends from the neighborhood of the capitular formen posteriorly to the level of the third or fourth coxae. Each gland (fig. 1) is in the form of a lobulated mass of alveoli more narrowed anteriorly than posteriorly. Similar to those of other ticks, e. g., *A. persicus* (Heller, 1858; Robinson and Davidson, 1913B), *Orinthodoros moubata* (Samson, 1909), *Ixodes ricinus* (Bonnet, 1907; Samson, 1909), *Dermacentor,* (Douglas, 1943; Arthur, 1960), the salivary gland of *A. vespertilionis* is composed of two types of alveoli, referred to as the first and second. Alveoli of the second type (fig. 1, A′) are small and form a compact mass in the mesial side of the anterior two-thirds of the gland. The anterior end of this mass is comparatively large, blunt, and appears to be separated from the gland, whereas its posterior end is narrow and tapering. The remainder of the gland is formed of lobules of alveoli of the first type (A). The salivary duct (s.d.), which appears to emerge from the anterior end of the mass of alveoli of the second type, extends towards the capitular foramen (c.f), where it passes to the inside of the basis capituli and opens into the posterolateral ends of the buccal cavity. There is no marked difference between the shape of the gland in unfed and fed ticks.

Pharynx. The pharynx (figs. 1 and 2, ph.), a large muscular organ of the foregut, lies inside the basis capituli and connects anteriorly with the buccal cavity and posteriorly with the oesophagus. The structure of the pharynx is

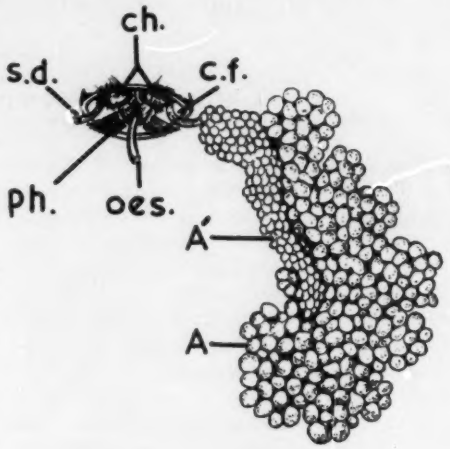

Fig. 1

FIGURE 1. Right salivary gland of *Argas* (*Carios*) *vespertilionis.* 12×. A, first type of alveoli; A′, second type of alveoli; c.f., capitular foramen; ch., base of chelicerae; oes., oesophagus; ph., pharynx; s.d., salivary duct.

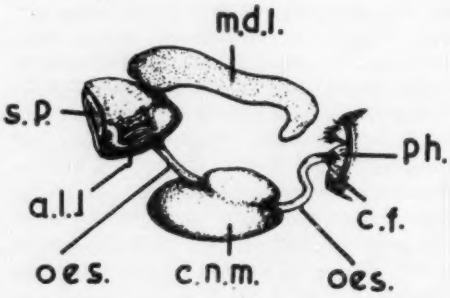

Fig. 2

FIGURE 2. Lateral view of the oesophagus, central nerve mass, and median dorsal lobe of the stomach. 14×. a.l.l., first anterolateral lobe of the stomach; c.f., capitular foramen; c.n.m., central nerve mass; m.d.l., median dorsal lobe of the stomach; oes., oesophagus; ph., pharynx; s.p., stomach proper.

similar to that of *A. persicus* as described by Robinson and Davidson (1913B).

Oesophagus. The oesophagus (fig. 2, oes.) is a narrow, delicate, S-shaped tube arising from the posterior extremity of the pharynx. It passes through the central nerve mass (c.n.m.), then extends dorsally to enter the stomach at the ventral surface of its anterior end.

Stomach. The stomach, with its branches, occupies the greater part of the body cavity. The median portion or stomach proper (fig. 3, s.p.), which gives rise to a series of branching

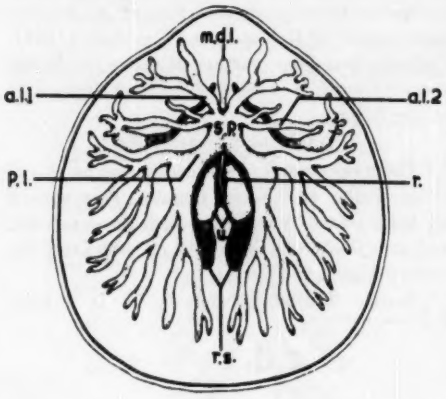

Fig. 3

FIGURE 3. Alimentary canal. approx. 4×. a.l.l., first anterolateral lobe; a.l.2, second anterolateral lobe; m.d.l., median dorsal lobe; p.l., posterolateral lobe; r., rectum; r.s., rectal sac; s.p., stomach proper; u., uterus.

lobes, is short, comparatively wide tube, situated medianly over the uterus in females or over the accessory gland in males. From the anterior end of the stomach proper arises a median dorsal lobe (m.d.l.), which extends anterodorsally to the central nerve mass, and bends ventrally to end near the capitular foramen. Close to the origin of this lobe arise two pairs of lateral lobes. The first pair (a.l.l) extends anteriorly and obliquely and each lobe divides into two branches, the anterior of which runs over the salivary gland and ends blindly in the anterolateral part of the body cavity, while the posterior branch runs laterally and ends with three small caecae. The second pair of lobes (a. 1.2) extends directly to the lateral margins of the body cavity. Each lobe, shortly after its origin, gives off a small branch that runs deeply ventrally and ends below the salivary gland. Laterally, the lobe gives off several small caecae, makes a retrograde curve, and extends ventrally and anteriorly to the uterus in females or accessory gland in males. It ends close to the central nerve mass.

From the posterior end of the stomach proper extends a pair of wider lobes (p.l.) each of which runs laterally for a short distance before dividing into outer and inner branches. The outer, narrower branch, shortly divides into two bifurcated branches, each terminating in two or more caecae. The inner, wider branch runs posteriorly over the lateral part of the uterus and oviducts in females or the vas deferens in males. It is divided into two branches, the outer of which redivides into two and ends in two small caecae. The inner one similarly divides, but only the mesial branch ends in two caecae.

Rectum. The rectum (tube communicatif of Blanc, 1910) (fig. 3, r.) is that portion of the alimentary canal that connects the stomach proper with the rectal sac (r.s.), although it belongs to the midgut. It is a funnel-shaped tube, longer than that of *A. persicus,* arising from the ventral surface of the posterior end of the stomach proper. It runs posteriorly and ventrally to open into the anteroventral surface of the rectal sac.

Rectal sac. The rectal sac (fig. 3, r.s.), a thin-walled, bilobed organ, lying ventrally in the middle of the body cavity, receives the openings of the Malpighian tubules close to that of the rectum. A short distance posterior of these openings the anal canal arises.

Anal canal. This is a short, delicate tube extending from the ventral surface of the rectal sac and running ventrally to the anus, which according to Hoogstraal (1958) is elliptical, situated at the midlength of the body, and guarded by two valves.

Reproductive System, Female

The genital organs of the female consist of ovaries, oviducts, uterus, vagina, and associated Gene's organ (fig. 4).

Ovaries. Generally, the ovary of ticks is single (Christophers, 1906; Samson, 1909; Robinson and Davidson, 1914; Lees and Beament, 1948; Roshdy, 1961). In unfed and fed adult females of *A. vespertilionis,* however, there are two ovaries situated ventrally and laterally of approximately the middle region of the body cavity. The two ovaries are connected together

with a very thin strand of tissue devoid of ova, and fine tracheoles, extending transversely below the two lobes of the uterus. Each ovary (fig. 4, ov.) forms a small, more or less round mass, the external surface of which is studded with ova at various stages of development. Only part of the posteroventral surface of the ovary is devoid of ova.

Oviducts. From each ovary an oviduct (ovd.), a narrow convoluted tube, extends anteriorly. It suddenly enlarges to form an elongated sac close to the anterior portion of the uterus. From this sac a narrow tube extends posteriorly close to the uterus, and enters the uterus in the middle of its lateral margin.

Uterus. The uterus (u.), the largest portion of the genital organs, occupies a median, ventral position in the body cavity. It consists of two large lobes which unite to form a median tubular neck. The lobes are partially hidden underneath the posterior lobes of the stomach. Between the two lobes of the uterus there is a narrow space through which the rectum passes. The anterior median neck leads to the vagina.

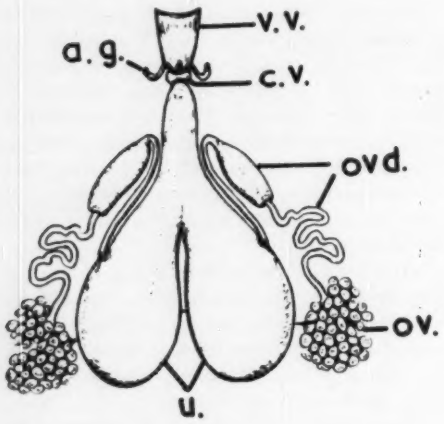

Fig. 4

FIGURE 4. Female genital organs. 6×. a.g., accessory gland; c.v., cervical portion of vagina; ov., ovary; ovd., oviduct; u., uterus; vv., vestibular portion of vagina.

Vagina. The vagina is a median ventral canal extending from the uterus to the external genital aperture. As in *A. persicus* (Robinson and Davidson, 1914), the vagina of *A. vespertilionis* consists of a distinct cervical and vestibular portion. The cervical portion (c.v.) is short and partially telescoped into the neck of the uterus. Anteriorly it joins the vestibular portion at its ventral surface. The vestibular portion (v.v) is long, thin-walled, and flat. It opens into the exoskeleton through the genital aperture. Into the posterolateral sides of the vestibular portion open into tubular accessory glands (a.g.), the posterior ends of which are coiled.

Gene's organ. Gene's organ, present in all female ticks, functions during oviposition (Nuttall et al, 1908; Robinson and Davidson, 1914; Lees and Beament, 1948; and others). In *A. vespertilionis,* this organ is similar to and occupies the same position as that of *A. persicus* as described by Robinson and Davidson (1914). Only the glandular portion of the organ in the former species is saeculated and not digitate as in the latter.

Reproductive System, Male

The genital organs of males occupy a position similar to those of females. They consist of testis, vasa deferentia, vesicula seminalis, and an ejaculatory duct with an associated accessory gland.

Testis. The testis (fig. 5, t.) is a thin,

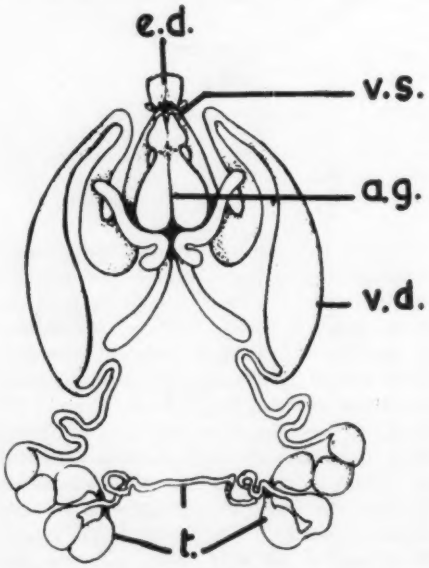

Fig. 5

FIGURE 5. Male genital organs. Approx. 6.5×. a.g., accessory gland; e.d., ejaculatory duct; t., testis; v.d., vas deferens; v.s., vesicular seminalis.

transverse tube resting on the anterior portion of the rectal sac. Laterally, the tube is coiled at its ends, convoluted and swollen. The swellings contain different stages of sperm development.

Vasa deferentia. Each lateral branch of the testis leads to the vas deferens (v.d.), which starts as a narrow coiled tube, running anteriorly, then widens into an elongated sac. Anteriorly, this sac narrows again and at the level of the accessory gland curves posteriorly and medianly to form a large U-shaped tube. The inner portion of this tube passes ventrad of the accessory gland. The vasa deferentia unit to form the vesicula seminalis.

Vesicula seminalis. This is a small triangular sac (figs. 5 and 6b, v.s.) lying below the anterior end of the dorsal lobe of the accessory gland and opening into the dorsal side of the ejaculatory duct.

Ejaculatory duct. The ejaculatory duct (figs. 5 and 6, e.d.) is the portion of the genital tract that leads to the outside of the exoskeleton through the genital aperture. It is wide, more or less flattened tube running ventrally below the central nerve mass. The posterior end of this duct receives the opening of the vesicula seminalis dorsally and the ducts of the accessory gland posteriorly, laterally and ventrally.

Accessory gland. The accessory gland has been described in different tick species (Heller, 1858; Christophers, 1906; Robinson and Davidson; 1914, Douglas, 1943, and others). Robinson and Davidson gave a full account of the structure of this organ in *A. persicus*. The gland consists of a number of lobes, some of which are spongy, others are granular.

The accessory gland of *A. vespertilionis* (fig. 6) is similar to that of *A. persicus* in position and number of lobes but differs in the shape of lobes. The anterior and posterior spongy lobes (a.s.l. and p.s.l.) are small tubular pairs attached basally to the ventrolateral sides of the posterior portion of the ejaculatory duct. The anterior lobes are smaller than those of *A. persicus,* while the posterior ones are comparatively long and extend posteriorly till the middle region of the median ventral granular lobe. The granular lobes are massive and consist of a median ventral lobe, three pairs of lateral lobes, an anterodorsal and a pair of posterodorsal lobes. The median ventral lobe (fig. 6a, m.v.l.) is small and oval, and joins the ejaculatory duct at its posterior end. The first lateral granular lobes (l.g.l.I) extend posteriorly from the lateral side of the median ventral lobe. The lobes are diverging and almost hidden beneath the vasa deferentia. The second, very small, pair of lateral lobes (l.g.l.II) extends posterior to the median ventral lobe.

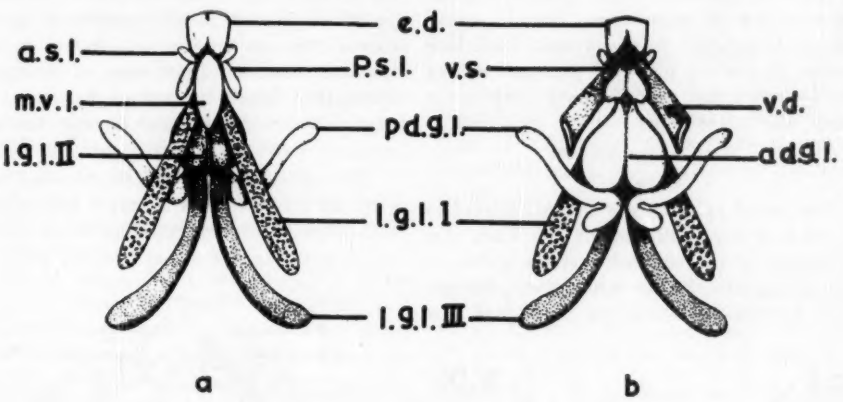

Fig. 6

FIGURE 6. Ventral (a) and dorsal (b) views of accessory gland of male. 20×. a.s.l., anterior spongy lobe; ad.g.l., anterodorsal granular lobe; e.d., ejaculatory duct; l.g.l. I–III, lateral granular lobes I–III; m.v.l., median ventral lobe; p.s.l., posterior spongy lobe; pd.g.l., posterodorsal granular lobe; v.d., vas deferens; v.s., vesicula seminalis.

The distal ends of these lobes are club-shaped. The third pair (l.g.l.III), the longest of all lateral lobes, arises from the lateral sides of the posterior region of the median ventral lobe, just dorsad to the second pair. Its diverging lobes extend posteriorly. The massive anterodorsal lobe (fig. 6b, ad.g.l.) completely covers the median ventral lobe, the second pair of lobes, and the bases of the first and third lobes as well as the lateral inner sides of the vasa deferentia. The small anterior part of this lobe overlies the vesicula seminalis; its end appears to be notched, whereas the posterior and major part of the lobe is swollen. The posterior end of the lobe is deeply notched by a median dorsal groove. The anterodorsal lobe joins the median ventral lobe at its dorsal side. The posterodorsal pair of granular lobes (pd.g.l.), which seems to join the anterodorsal lobe, is, unlike that of *A. persicus,* long and bent. Each lobe consists of a short posterior part and a long anterior one which runs obliquely towards the lateral side of the gland.

Respiratory System

Spiracles

The paired spiracles are situated laterad to the coxae of the fourth pair of legs. Each (fig. 7) consists of two elevated portions which enclose a crescentic slit, the ostium (os.). The anterior portion, the spiracular plate (s.pl.), is semi-circular and convex. The convex surface is membraneous and apparently porous. In whole mount preparations, a number of chitinised pillars or pedicels (p.) can be seen through the outer surface. The posterior portion, the macula (M), is a more or less round area of hard sculptured cuticle. Spiracles of argasid ticks are basically similar (Robinson and Davidson, 1913b; Mellanby, 1935; Browning, 1954). The anterior area, however, has been a matter of dispute among these authors. Robinson and Davidson showed that the anterior area of the spiracle of *A. persicus* is perforated by communicating pores with interpedicellar spaces which lead to the cavity of the atrium. They referred to this part as "area porosa." In *O. moubata,* Mellanby (1935) described a similar perforated area which he termed "cribiform plate." Browning (1954) considered the apparent pores on the external surface as nothing but expanded distal ends of the pillars where they joined the surface membrane. He applied the term spiracular plate to this area.

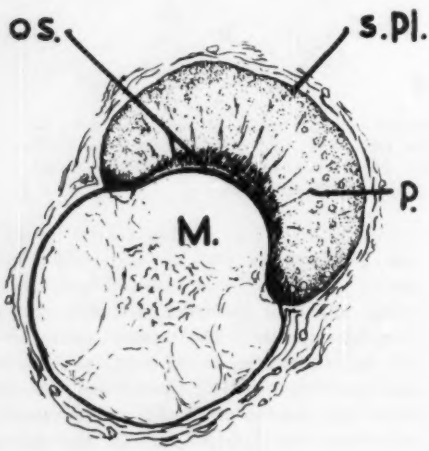

Fig. 7

FIGURE 7. Spiracle. Approx. 140×. M., macula; os., ostium; p., pillars; s.pl., spiracular plate.

Tracheae

The spiracle leads into a short atrium which gives off three tracheal trunks arising close to each other. These are referred to as the anterior, posterior and dorsal trunks (fig. 8).

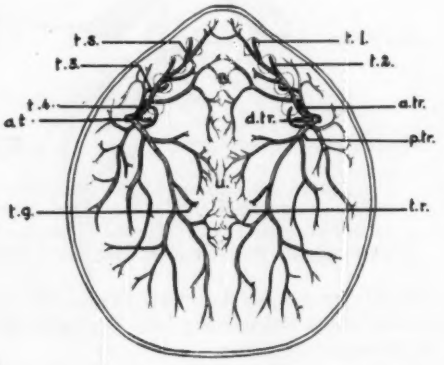

Fig. 8

FIGURE 8. Tracheal system Approx. 4×. a.tr., anterior trunk; at., atrium; B., place of the central nerve mass; d.tr., dorsal trunk; p.tr., posterior trunk; t.1–4, tracheae to legs 1–4; t.g., trachea to gonad; t.r., trachea to rectal sac; t.s., trachea to salivary gland; U., place of uterus.

Anterior trunk. This trunk (a.tr.) extends anteriorly, more or less parallel to the outer margin of the body. At its beginning, the trunk

gives off a small ventrolateral branch, which runs inwards to supply the central nerve mass and the anterior region of the genital organs. It also gives off a trachea to the third leg (t.3) and ends with several brancheoles in the ventral side of the anterolateral region of the body cavity. The main anterior trunk gives off a trachea to the salivary gland (t.s.), another to the second leg (t.2), and a third to the first leg (t.1). It ends with fine brancheoles at the anterior part of the body cavity. The two trunks are connected by a small trachea surrounding the capitular foramen.

Posterior trunk. The posterior trunk (p.tr.) extends posteriorly, obliquely inwards, and after a short distance gives off a trachea to the fourth leg (t.4) from one side and other branch from the other side. This latter branch runs posteriorly and laterally and divides into several branches to supply the muscles and ventral body wall in this region. The trunk then gives off an inner branch to supply the genital organs and alimentary canal branches. The trunk then continues its course and gives off trachea to the gonads (t.g.) and rectal sac (t.r.), and ends with long dividing branches to supply muscles and posterior extremities of the gut branches.

Dorsal trunk. This trunk (d.tr.) arises from the atrium, between the other trunks and extends dorsally, passing through the notch between the second anterolateral lobe and the posterolateral lobe of the stomach proper. On the dorsal surface of the body cavity, the trunk divides into different branches running anteriorly and posteriorly to supply the heart, Malpighian tubules, and branches of the gut.

SUMMARY

The morphology of the digestive, reproductive, and respiratory systems of *Argas vespertilionis*, representing the subgenus *Carios*, is described and compared with that of *A. persicus* representing the subgenus *Argas*.

Anterolateral lobes from the median portion of the stomach number one pair in *persicus* and two pairs in *vespertilionis;* in the latter species a ventral branch of the second lobe extends anteriorly and ends close to the central nerve mass. A median dorsal lobe arising from the anterior extremity of the stomach proper is present in *A. vespertilionis* but not in *A. persicus*. The rectum is comparatively longer in the former species than in the latter.

In unfed or fed females of *A. vespertilionis*, two ovaries, connected together with a very thin strand of tissue and tracheoles, are disposed laterally beside the bilobed uterus, but there is only one transversely located ovary in *A. persicus*. A prominent swollen, sac-like, portion of the oviduct present in the former species is absent in the latter.

The structure of the genital organs of mature males of both species is similar except that the lobes of the accessory gland in *vespertilionis* vary in shape and length.

Three main tracheal trunks arise from the atrium of the spiracle of *A. vespertilionis*, whereas in *A. persicus* they number three or five.

ACKNOWLEDGMENT

The writer is greatly indebted to Dr. H. Hoogstraal, Head of Medical Zoology Department of NAMRU-3, for valuable suggestions during this study and for reading the manuscript.

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ENDOPARASITIC CHIGGERS: I. CHIROPTERA, A NEW HOST ORDER FOR INTRANASAL CHIGGERS, WITH DESCRIPTIONS OF TWO NEW GENERA AND SPECIES (ACARINA: TROMBICULIDAE)

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Infestation of the nasal passages of vertebrates by chiggers was first recorded in 1953 by Fain and Vercammen-Grandjean, in Congolese rodents. Later, intranasal chiggers were found in Malaysian rodents (Audy, 1954; Audy and Nadchatram 1957). Southcott (1957) described a new genus of chigger from the repiratory passages of a Pacific sea snake. It has become obvious, partly due to the efforts of such diligent workers as Fain, that to ignore the intranasal niche in routine collection of parasitic Acarina is to overlook a major focus of infestation and, possibly, of disease transmission. Consequently, when the opportunity arose to examine a collection of nearly 300 bats from caves in Panama, they were examined intranasally as well as externally. Results of this examination are reported here.

MATERIALS AND METHODS

Two hundred and eighty-seven bats, representing 11 genera, destroyed in Chilibrillo Caves, Chilibre, Panama, during a bat-reduction campaign, were made available to us for ectoparasite examination. After ectoparasites were removed, the nasal passages of the bats were exposed and turbinates and sinuses scanned with the aid of a binocular dissecting microscope.

Subsequently, another collection of 16 bats was made from a culvert near Paraiso, Canal Zone. These were examined by a nasal-washing technique described elsewhere (Yunker, in press), after which the nasal passages were exposed and checked for remaining mites. Some mites were cleared in trypsin digestant solution (Newell, 1947), mounted on microslides in Hoyer's modification of Berlese's medium, and identified with phase-contrast microscopy. Others were mounted directly on microslides in polyvinyl alcohol medium. In the following descriptions abbreviations of scutal measurements and descriptive terminology follow those proposed by Wharton et al (1951).

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Alexfainia gen. n.

Description: Trombiculinae; sensillae flagelliform; all legs with seven segments; telofemora and genua of all legs each with a heavy, serrate seta; palpal femur unusually large, bearing ventral hooklike processes (hamuli); palpal claw with a single hooklike tine; cheliceral base reduced; dorsal body setae nude; eyes absent; habitat intranasal in Chiroptera. Monotypic: *Alexfainia chilonycteris* sp. n.

Remarks: Specimens belonging to this genus will key to *Trombicula* in the keys of Wharton and Fuller (1952, p. 41) and Brennan and Jones (1959, p. 10), but they are readily separable on the basis of the heavy serrate setae of the legs, the hamuli of the palpal femur, the heavy palpal femora, the absence of eyes, the reduced cheliceral bases, and the single-tined palpal claw. The new genus is named after Dr. Alex Fain, Institut de Médecine Tropicale Prince Léopold, Antwerp, Belgium, in recognition of his many striking discoveries of endoparasitic Acarina.

Alexfainia chilonycteris sp. n.

Description of larval holotype: Idiosoma: large, 1220 microns by 810 microns; elongate and oval in shape. Eyes absent. Dorsal setal formula 2-12-6-7-6-3-2, total setae 38; all thin, apparently nude; humeral setae approximately 55 microns long, anterior dorsal setae approximately 65 microns long and posterior dorsal setae approximately 60 microns long. Ventral setal formula 2-2-4-6-9-4 . . . , total 35; sternal setae nude, approximately 55 microns long; all remaining ventral setae thicker than dorsal setae, with obvious setules, and decreasing in size posteriorly; anterior ventral setae approximately 50 microns long, and posterior ventral setae approximately 45 microns long. Anus a simple slit situated at fourth row of ventral setae (excluding sternals). Scutum roughly trapezoidal, narrow, and concave anteriorly, widening posteriorly, and joining straight posterior margin at broadly rounded angles; densely punctate over entire surface; a transverse depression between sensillary

bases; three anterior setae, two posterior setae and two sensillae; anterolateral setae short and nude; anteromedian seta slightly longer and apparently nude; posterolaterals longer than anterolaterals and with minute setules; sensillae flagelliform, arising from widely separated bases situated in anterior half of scutum, tapering distally to latent terminations, with indistinct setules on basal portion. (Sensillae broken on holotype; their terminations described from paratypes.) Scutal measurements in microns: AW 75, PW 130, SB 80, ASB 35, PSB 47, AP 55, AM 20, AL 13, PL 40, S 84 (the last structure incomplete).

Gnathosoma: Gnathosomal base, cheliceral base, and all segments of palpi densely punctate. Palpal coxae each with a single, short, nude, ventral seta. Palpal femur greatly enlarged, with a single, short, nude, dorsal seta, and three recurved hamuli, the latter arising from a common base on the ventrolateral apical aspect of femur. Genu short, broad, with a single, nude, dorsal seta somewhat longer than those of coxa and femur. Tibia short, with three nude setae similar to that of genu, and a heavy claw without accessory prongs. Tarsus small, with a tarsala, a subterminala, two long, heavy, densely setulose setae, and five short, bare setae. Cheliceral base reduced, more than four times longer than wide; cheliceral blade curved, with a fine pointed tip and a single, subapical, dorsal tooth. Galea reduced, with a slender, attenuate, nude seta.

Legs: All legs with seven segments, paired claws, and a clawlike empodium. I-Coxa, trochanter, and basifemur each with a single nude seta. Telofemur with three, moderately long, nude setae, one elongate, thin seta bearing minute barbs, and a thick, elongate, attenuate, serrate seta. Genu with two moderately long, attenuate, nude setae, two blunt genualae (20 microns), a minute microgenuala, an elongate, thin seta bearing minute barbs, and a thick, elongate, attenuate, serrate seta. Tibia with seven moderately long, nude setae, a single branched seta of similar size, two tibialae (23 microns), and a short, blunt microtibiala. Tarsus with 17–19 branched setae, four nude setae, a basal tarsala (33 microns), a minute medial microtarsala, a distal subterminala (28 microns) closely associated with a thinner, shorter parasubterminala (15 microns) and a pretarsala (22 microns). Urstigma present on posteroapical margin of coxa I (largest diameter 16 microns).

II-Coxa and trochanter each with a single nude seta. Basifemur with two nude setae. Telofemur with two moderately long, nude setae, a longer attenuate seta that bears minute barbs, and a thick, short (31 microns), blunt serrate ventral seta that resembles the basal half of the serrate seta of telofemur I. Genu with one elongate, attenuate, nude, seta, and a similar one bearing short barbs, a genuala (17 microns), and a thick, short (35 microns), blunt, serrate ventral seta that resembles that of the preceding segment. Tibia with three branched and three nude setae and two tibialae (18 and 21 microns); tarsus with 10 to 12 branched setae, three moderately long, nude setae, a basal tarsala (22 microns), a minute, basal microtarsala, and a pretarsala (22 microns).

III—Similar to leg II with the following exceptions: one nude seta rather than two on telofemur; genuala 20 microns long; one tibiala (20 microns) rather than two; tarsus with 16 to 18 branched setae and no modified setae.

Type material: Holotype (RML No. 36602) and eight paratypes from *Chilonycteris rubiginosa fusca* Allen; Panama, Canal Zone, 1.5 mi N. of Paraiso; 18 August 1960; coll. C. E. Yunker. Sixty-three paratypes from same host; Panama, Chilibre, Chilibrillo Caves, 2 August 1960, coll. N. Gale. One paratype from *Carollia perspicillata azteca* Saussure, same data. Holotype and 30 paratypes in Rocky Mountain Laboratory; remainder of paratype material to be divided among U. S. National Museum, British Museum (Natural History), Institute of Acarology of the Ohio State University, and Chicago Natural History Museum.

Other material includes 30 alcoholic specimens from type host, Panama, Chilibre, Chilibrillo Caves, 2 August 1960.

Remarks: Body size of paratype specimens varies with degree of engorgement, from 606 microns to 1840 microns long. Most of the specimens are over 1300 microns in length; few are under 900 microns. Some paratype specimens clearly show small barbs or setules on sensillae and on all scutal setae except anterolaterals. The sensillae of most specimens are broken off; in the few that had intact sensillae, these structures were seen to be extremely attenuate, and their terminations could not be defined with oil-immersion magnification. A similar attenuation was noted in the galeal setae. Of 10 *Chilonycteris rubiginosa fusca* examined from Chilibrillo Caves, all were infested with numbers of chiggers varying from 9 to 18, with an average of 10 per host. Of the same species of bat from a culvert in Paraiso, only 4 out of 16 were positive, and these never harbored more than 6 per host. The single chigger from *Carollia perspicillata azteca* reflects its incidence in 32 of these bats from Chilibrillo Caves.

Vergrandia gen. n.

Description: Trombiculinae; sensillae flagelliform; all legs with seven segments; palpal claw with a single hooklike tine; cheliceral bases reduced; body setae all vested; eyes reduced to a single minute pair; habitat intranasal in Chiroptera. Monotypic: *Vergrandia galei*, new species.

Remarks: Three other genera of the subfamily are characterized by a single-tined palpal claw: *Crotiscus* Ewing, 1944, *Kaaia* Brennan, 1958, and *Alexfainia* of this paper. Characters that distinguish *Vergrandia* from *Crotiscus* and *Kaaia* are reduction of eyes; nude anterolateral and anteromedian scutal setae and sensillae; sensillary bases of scutum close-set and vaginate; palpal tarsus with one branched and six nude unmodified setae; cheliceral bases re-

duced; and an intranasal habitat. In addition to certain of these characters, *Vergrandia* differs from *Alexfainia* in the presence of eyes, absence of heavy serrate setae on telofemora and genua of all legs, absence of hamuli on palpal femur; presence of pilosities on dorsal body setae; and shape and configuration of scutum. The name *Vergrandia* is an arbitrary combination of letters (treated as masculine) chosen from the surname of Dr. P. H. Vercammen-Grandjean who first reported, with Fain, the intranasal habitat for chiggers.

Vergrandia galei sp. n.

Description of larval holotype Idiosoma: large, 1310 microns by 825 microns: elongate and oval in shape. A single, minute eye (5 microns dia.) present on either side of scutum at level of anteromedian seta. Dorsal setal formula indistinguishable, total setae approximately 44; all vested; a single pair of humeral setae about 54 microns long, anterior dorsal setae about 60 microns long, and posterior dorsal setae about 50 microns long. Ventral setae 2–2 (sternals) plus approximately 34; all vested; increasing in size posteriorly; anterior sternal setae about 38 microns long, anterior ventral setae about 30 microns long, and posterior ventral setae about 46 microns long. Anus ventral. Scutum roughly rectangular; anterior margin narrower than posterior, with a slight median convexity; lateral margins concave; posterior margin convex, with a slight median invagination; entire surface punctate, more densely so posteriorly; sensillary bases close-set, vaginate in knoblike projections of scutum; with three nude anterior setae, two branched posterior setae, and two nude flagelliform sensillae. Scutal measurements in microns: AW 36, PW 50, SB 23, ASB 30, PSB 22, AP 34, AM 47, AL 22, PL 39, S 71.

Gnathosoma: Gnathosomal base, palpal femur and genu densely punctate. Palpal coxae each with a single, branched, ventral seta. Femur large, with a single, branched, dorsal seta. Genu with a single, nude, dorsal seta. Tibia with three nude setae and a heavy claw without accessory prongs. Tarsus with a tarsala, a subterminala, one branched seta and six nude setae. Cheliceral base reduced, longer than wide; cheliceral blade strongly curved, with a single, subapical, dorsal tooth. Galea reduced, with a nude seta (galeal seta seen only on paratype specimens).

Legs: All legs with seven segments, paired claws, and a clawlike empodium. I-Coxa, trochanter, and basifemur each with a single branched seta. Telofemur with five branched setae. Genu with four branched setae, three genualae (18 microns), and a short, thick microgenuala. Tibia with eight branched setae, two strong tibialae (23 microns), and a short, thick microtibiala. Tarsus with 17 to 19 branched setae, a heavy, long (33 microns), basal tarsala, a short, blunt, medial microtarsala, a distal subterminala (23 microns) closely associated with a shorter (14 microns), thinner parasubterminala, and a pretarsala (17 microns). Urstigma present on posteroapical margin of coxa I (largest diameter 11 microns).

II - Coxa and trochanter each with a single branched seta. Basifemur with two branched setae. Telofemur with four branched setae. Genu with three branched setae and a genuala (19 microns). Tibia with six branched setae and two tibialae (16 microns), tarsus with 15 and 16 branched setae, a basal tarsala (20 microns), a short, blunt microtarsala, and a pretarsala (17 microns).

III — Similar to leg II with the following exceptions: telofemur with three rather than four branched setae; genuala 24 microns; one tibiala (26 microns) rather than two; tarsus with 14–16 branched setae and no modified setae.

Type material: Holotype (RML No. 36602) and two paratypes from *Chilonycteris rubiginosa fusca* Allen; Panama; Chilibre, Chilibrillo Caves; 2 August 1960; coll. N. Gale. Holotype and one paratype in Rocky Mountain Laboratory; one paratype in U. S. National Museum.

Remarks: These mites were taken from nasal passages of bats that also harbored *Alexfainia chilonycteris*. The species is named in honor of the collector of the bats, Dr. Nathan Gale, Division of Veterinary Medicine, Health Bureau, Canal Zone, whose enthusiastic cooperation is greatly appreciated.

TROMBICULINAE, undetermined species

A single specimen, representing a third species, was collected from the nasal passages of the *Chilonycteris* from Chilibrillo Caves. Many diagnostic features, however, are missing, including gnathosoma and scutum, and identification is not possible. The body is large (1100 microns long by 420 microns wide), with a single pair of lenslike eyes (11 microns dia.). All body setae are vested; those of dorsum are approximately 50 in number; humeral setae were not seen; ventral setae are approximately 38 in number, starting 2–2–8–2–4 . . . . The legs are short and their segments are wide in relation to length. All legs are composed of seven punctate segments, but the femoral divisions of II and III are barely discernible. Setation of the legs is as follows: coxae and trochanters of all legs with a single branched seta. I-Basifemur with one (?) branched seta; telofemur with five branched setae; genu with four branched setae, three genualae (17 microns), and a minute microgenuala; tibia with eight branched setae, two tibialae (20 microns), and a small microtibiala; tarsus with 16 to 19 branched setae, a heavy, long (30 microns) tar-

sala, a short microtarsala, a subterminala (16 microns) (parasubterminala if present not seen), and a pretarsala (16 microns). II-Basifemur and telofemur as in leg I; genu with three branched setae and a genuala (17 microns); tibia with six branched setae and two tibialae (17 microns); tarsus with 14 to 16 branched setae, a heavy tarsala (16 microns), a short microtarsala, and a pretarsala (16 microns). III-Basifemur with two branched setae; telofemur with three branched setae; genu with three branched setae and a genuala (19 microns); tibia with six branched setae and a tibiala (17 microns); tarsus with 12 or 13 branched setae. All tarsi end in paired claws and a clawlike empodium. An urstigma is present on the posteroapical margin of coxa I (largest diameter 6 microns). This species may be distinguished from *Vergrandia galei* by its larger eyes, shorter genuala III, and the general appearance of its short, thick leg segments. Tarsus III of this species is 65 microns long from joint to base of claws, while that of *V. galei* is 90 microns long.

SUMMARY

The intranasal niche as a habitat for chiggers, previously observed in rodents, obtains for Chiroptera. Two new genera and species of Trombiculidae, *Alexfainia chilonycteris* and *Vergrandia galei*, are described from the nasal passages of *Chilonycteris rubiginosa fusca* Allen. A third unidentifiable species from this host is described from a single incomplete specimen.

ACKNOWLEDGMENTS

The authors are grateful to Major V. J. Tipton, Environmental Health Branch, Office of the Surgeon General, U. S. Army Caribbean, who provided assistance during the collection of certain of the bats, and to Dr. J. M. Brennan, Middle America Research Unit, National Institutes of Health, who gave advice.

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Explanation of Plates

PLATE I. *Alexfainia chilonycteris* sp. n. *a*, gnathosoma, ventral aspect (left) and dorsal aspect (right); *b*, dorsum; *c*, scutum; *d*, legs I and II, ventral aspect; *d'*, leg I, specialized setae; *d''*, leg II, specialized setae; *e*, leg III ventral aspect.

PLATE II. *Vergrandia galei* sp. n. *a*, gnathosoma, dorsal aspect; *b*, palpal tibia and tarsus, dorsal aspect; *c*, body setae,—1, anterior dorsal seta, 2, anterior ventral seta; *d*, scutum; *e*, *f*, *g*, specialized setae of legs I, II, and III, respectively.

PLATE I

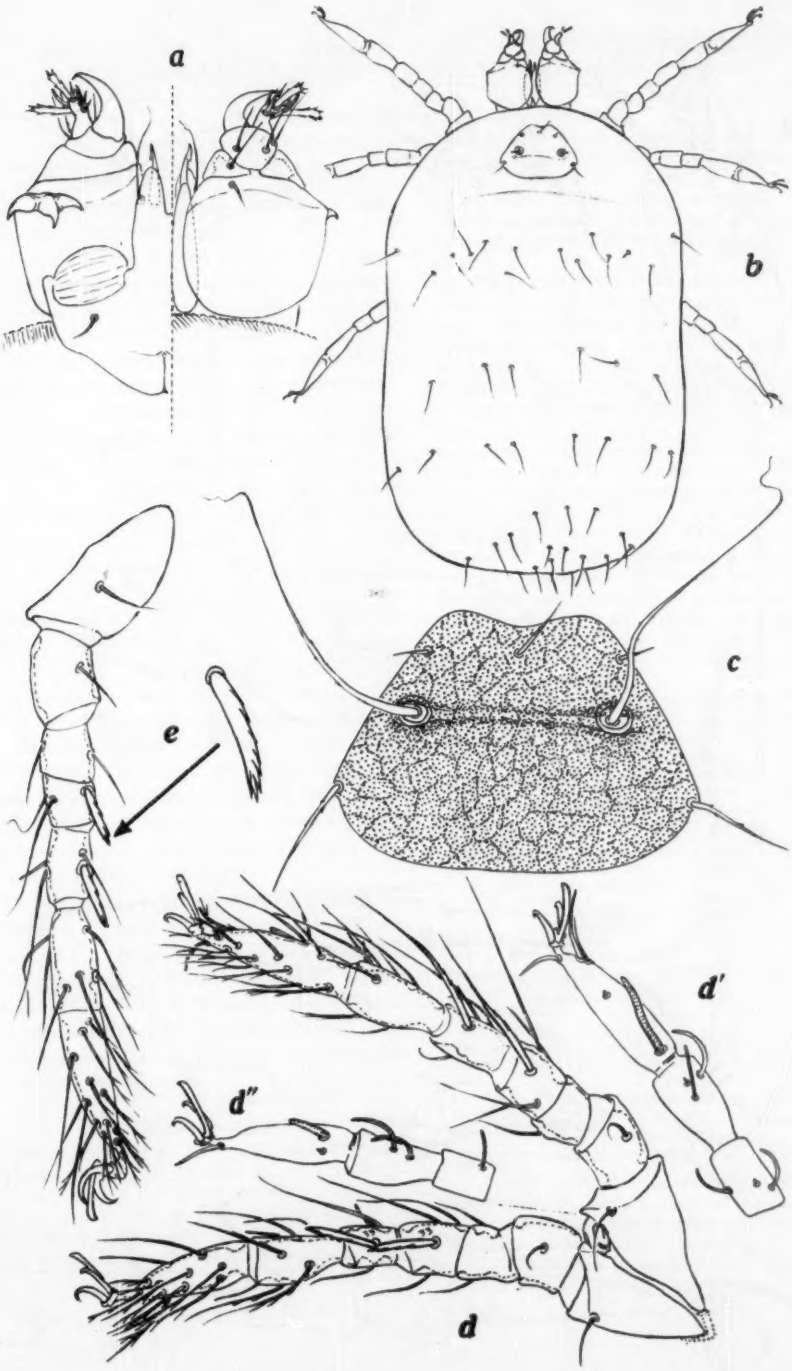
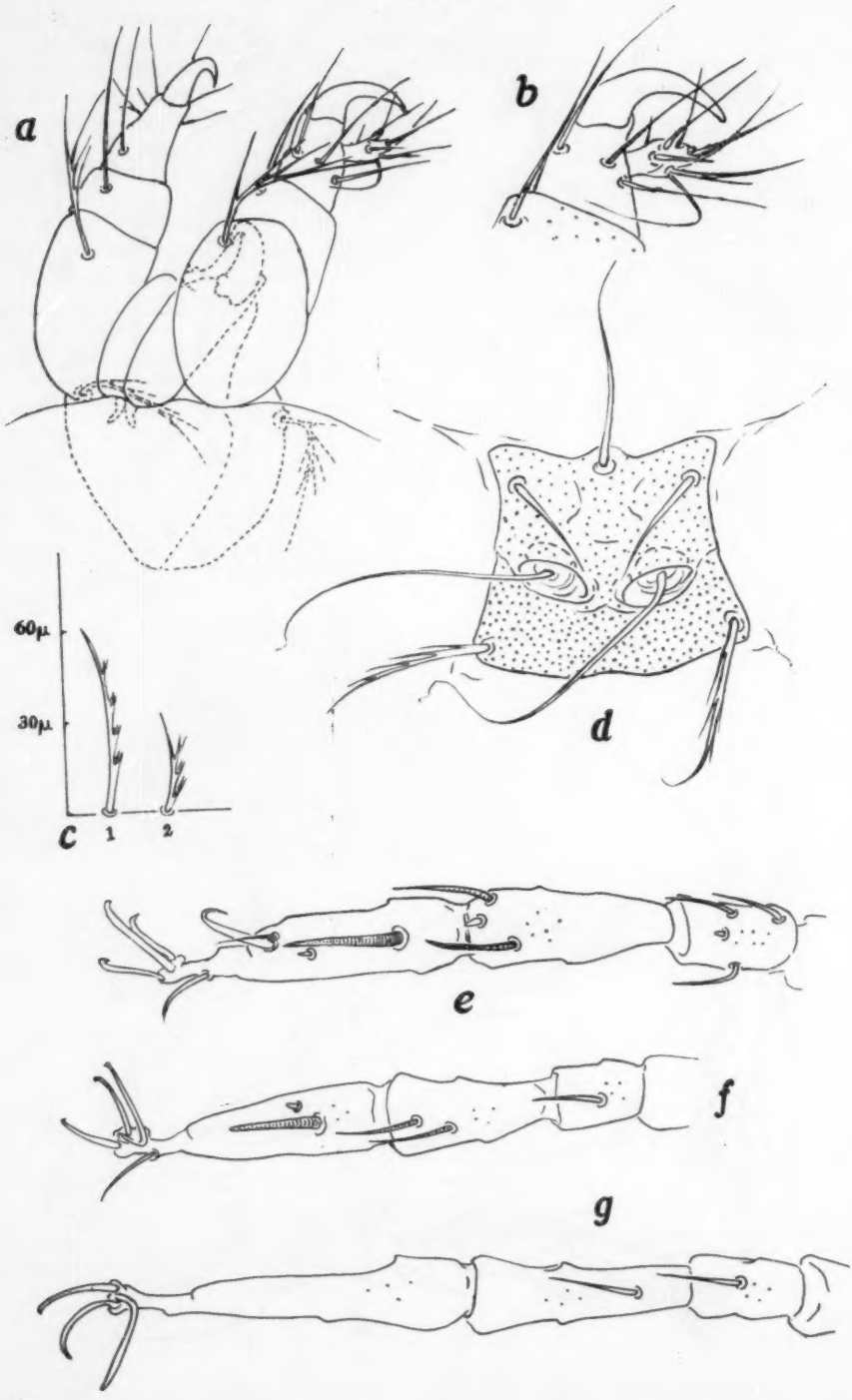


PLATE II



DRUG-RESISTANCE IN *EIMERA TENELLA*. I. THE EXPERIMENTAL DEVELOPMENT OF A GLYCARBYLAMIDE-RESISTANT STRAIN

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T. compounds currently used as prophylactics for avian coccidiosis are employed at levels that control the severity of the disease without completely suppressing the parasites. This permits some of the organisms to complete their life-cycle and a resultant immunity to develop. The continuous feeding of anticoccidial compounds to growing chickens, in this manner, presents an ideal situation for the development of drug-resistant strains of coccidia. Although the ability of microorganisms to develop a tolerance for drugs was recognized almost simultaneously with the advent of specific chemotherapy, little has been published on this aspect of the chemical control of avian coccidiosis.

Horton-Smith (1951) first suggested the possible development of drug-fast strains. Following this, Harwood and Stunz (1953) and Cuckler and Malanga (1955) studied field strains for evidence of drug resistance. Cuckler and Malanga (1955), Joyner (1957), and Horton-Smith (1958) developed drug-fast strains under laboratory conditions. The criteria used by these investigators for assessing drug resistance varied from increased oocyst production to increased mortality.

The foregoing reports, together with the continuing reliance on chemicals for the prevention of the disease, suggested that this aspect of the chemotherapy of coccidiosis merited further study. The data herein reported are those obtained from the first of a planned series of studies on the development of drug-fast strains of *Eimeria tenella*.

MATERIALS AND METHODS

Development of a drug-fast strain. A total of 1,565 coccidia-free, mixed-breed chicks, 3 weeks old, were used in this portion of the study. The birds were grouped by weight (*see* Gardiner and Wehr, 1950) into four groups for the first and into seven groups for each of the 11 remaining passages of the series. Each group usually contained 19 to 21 chicks; but because of a shortage of birds, some of the control groups of the third passage contained only 10.

The parent strain of *E. tenella* has been propagated in this laboratory for many years and has never been exposed to any drug. In the course of the following experiments, two lines of oocysts were developed from the parent strain. One, designated "resistant," was passed through chicks that had continuous low-level glycarbylamide intake. The other, designated "normal," was passed an equal number of times through birds that received no medication. The latter strain had a dual purpose. It served as a reference standard for the response of any group of chicks to an infection, and as a check that the virulence of the strains was not increased by the repeated, rapid passages through the hosts. Thus, any decrease in the sensitivity of the test strain, not reflected in the normal strain, was presumed to be due to drug tolerance.

Table I lists the various groups and the regimen followed for all except the first passage.

TABLE I. *Groups and experimental conditions for passages 2 through 12.*

Group No.	Group designation	Percent glycarbylamide in ration*	Group of preceding passage from which inoculum originated
A	Resistant, medicated	0.0015	A
B	Resistant, medicated	0.003	A
C	Resistant, unmedicated	None	A
D	Normal, medicated	0.003	E
E	Normal, unmedicated	None	E
F	Uninoculated, medicated control	0.003	No inoculation
G	Uninoculated, unmedicated control	None	No inoculation

* When this study was initiated the recommended level of glycarbylamide was 0.003 percent. Subsequently, it was increased to 0.006 percent.

The initial passage consisted of only four groups: A, B, E, and G. Each bird of the first three groups received 50,000 sporulated *E. tenella* oocysts.

Group A, which received only 0.0015 percent glycarbylamide, was necessary at the start in order to recover enough oocysts from medicated birds to inoculate those of the succeeding passages and served this purpose throughout. Oocysts from group E were used to inoculate the birds of the normal groups.

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The birds of the medicated groups were started on mash containing the appropriate level of glycarbylamide 24 hours prior to inoculation of the oocysts. In all but the first passage, the inoculation consisted of 100,000 *E. tenella* oocysts per bird. All surviving birds were weighed and necropsied on the eighth day following inoculation. At this time the cecal lesions were evaluated and the pathologic index (*see* Lynch, 1961) calculated for each group. Oocyst cultures were prepared, according to the method of Gardiner and McLoughlin (1960), from each of the inoculated groups and were allowed to stand for 5 days at room temperature to permit sporulation. At the end of this period the oocysts were washed, concentrated, and counted. The total number of oocysts per culture was divided by the number of birds used in its preparation to give an average number of oocysts recovered per bird. This, in turn, was divided by the number of oocysts inoculated per bird and the resultant figure recorded as oocysts recovered per oocyst inoculated.

Tests for cross-resistance. Two trials used 441 coccidia-free chicks, 3 to 4 weeks old. The inocula were resistant- and normal-line oocysts from the twelfth serial passage. In the first trial, group comparisons were made on the response to 0.0125 percent nicarbazin, 0.06 percent Trithiadol, 0.0125 percent zoalene, 0.0055 percent nitrofurazone, 0.002 percent arsenosobenzene, and 0.06 percent Unistat. (In all tests, the percentage figure indicates the amount of active ingredient(s) in the finished feed.) At the same time, tests were also made on the uninoculated, unmedicated control, the inoculated, unmedicated control, and the 0.003 percent glycarbylamide-medicated groups. The second trial used only nitrofurazone-medicated groups and the usual control groups.

In the tests for cross-resistance, the birds were distributed on a weight basis as before into groups of 19 to 21 birds. The experimental groups as well as the glycarbylamide-medicated controls were started on the appropriate medicated mash 24 hours prior to inoculation with 100,000 *E. tenella* oocysts. One group on each medication and an inoculated, unmedicated control group received normal-line oocysts; similar groups received resistant-line oocysts. On the fourteenth day after inoculation, the surviving birds were weighed, necropsied, and the cecal lesions evaluated.

RESULTS

Development of a drug-fast strain. As judged by the morbidity and mortality of the inoculated, unmedicated control groups, the infections resulting from the inoculation of normal- or resistant-line oocysts were of approximately equal intensity in any given passage. Variation in the intensity of infection from passage to passage did not appear to be reflected in the response of the medicated groups. Even in instances where the morbidity and mortality reached a peak in the normal, unmedicated control groups, birds given a similar number of the same oocysts were completely protected against mortality, and morbidity was adequately controlled by 0.003 percent glycarbylamide. Beginning with the ninth passage, the mortality rate was greater in both the normal and resistant unmedicated groups than it had been in the preceding passages. However, glycarbylamide afforded complete protection from mortality in the groups inoculated with the normal *E. tenella*. The drug failed to provide this protection to the birds that were inoculated with the resistant oocysts.

The uninoculated, unmedicated control group (G) and the uninoculated, medicated control group (F) permitted comparisons of rate of weight gain. In general, the rate of gain of group G and the 0.003 percent glycarbylamide-medicated groups receiving either normal or resistant oocysts followed the same trend through the eighth passage. From the ninth through the twelfth passages, group G and the normal medicated groups continued to follow a parallel pattern; but the rate of gain of the resistant medicated groups diminished considerably.

The pertinent data obtained from each passage for the 0.003 percent glycarbylamide-medicated groups that received normal- and resistant-line oocysts, respectively, are presented in table II. The table shows that, initially, oocyst production was low in both groups receiving 0.003 percent glycarbylamide. Oocyst production by the resistant groups showed considerable fluctuation in the various passages; but beginning with the third passage it exceeded that of the corresponding normal groups.

The growth data and pathologic indices for the resistant and normal medicated groups are presented graphically (figs. 1, 2).

Cross-resistance. The pertinent data are presented in table III.

Among the medicated experimental groups in the first trial, only those given nitrofurazone had a detectable difference in their response to the normal or to the glycarbylamide-resistant strain of *E. tenella*. Nitrofurazone afforded complete protection from mortality against the normal strain but was less successful against the glycarbylamide-fast strain. Similar results were obtained in a second trial that consisted of only nitrofurazone-medicated groups and the usual unmedicated controls. There was approxi-

TABLE II. *A comparison of the effects in glycarbylamide-medicated birds resulting from a single inoculation of 100,000 normal- or resistant-line oocysts of* Eimeria tenella.

Passage No.	Inoculum	Birds			Pathologic index	Oocysts recovered per oocyst inoculated	Mean weight gain per bird started		Mean weight gain per surviving bird	
		No. started	Deaths No.	Deaths Pct.			Grams	Pct. UUC*	Grams	Pct. UUC*
2	Normal	19	0	0	0.53	4	72	85	72	85
	Resistant	19	0	0	0.42	3	77	91	77	91
3	Normal	10	0	0	1.00	13	137	111	137	111
	Resistant	10	0	0	2.10	100	125	102	125	102
4	Normal	20	0	0	0.55	2	128	102	128	102
	Resistant	20	0	0	0.35	3	137	110	137	110
5	Normal	20	0	0	0.05	2	192	110	192	110
	Resistant	20	0	0	0.60	8	191	110	191	110
6	Normal	20	0	0	0.00	2	166	99	166	99
	Resistant	20	0	0	1.70	194	154	92	154	92
7	Normal	21	0	0	0.33	18	176	89	176	89
	Resistant	20	0	0	2.30	355	183	93	183	93
8	Normal	20	0	0	0.30	16	109	94	109	94
	Resistant	20	0	0	1.85	120	112	97	112	97
9	Normal	20	0	0	0.30	33	122	88	122	88
	Resistant	20	0	0	2.20	139	86	62	86	62
10	Normal	19	0	0	0.16	19	117	82	117	82
	Resistant	20	1	5	2.40	206	60	42	73	51
11	Normal	20	0	0	0.40	71	126	91	126	91
	Resistant	20	3	15	2.70	133	9	6	39	28
12	Normal	20	0	0	0.10	27	110	94	110	94
	Resistant	20	3	15	2.45	47	28	24	63	54

* Percent of the mean weight gain of the uninoculated, unmedicated control group for the particular passage.

TABLE III. *Results of tests for cross-resistance.*

Group	Inoculum	Birds			Pathologic index	Mean weight gain			
		No. started	Deaths No.	Deaths pct.		Per bird started		Per surviving bird	
						Grams	Pct. UUC	Grams	Pct. UUC
TRIAL NO. 1									
Uninoculated, unmedicated control	None	21	0	0	0.00	223	100	223	100
Inoculated, unmedicated control	Normal	20	4	20	2.35	83	37	171	77
Inoculated, unmedicated control	Resistant	20	5	25	2.50	72	32	171	77
Glycarbylamide medicated	Normal	20	0	0	0.05	226	101	226	101
Glycarbylamide medicated	Resistant	20	4	20	2.40	92	41	177	79
Nicarbazin medicated	Normal	20	0	0	0.00	223	100	223	100
Nicarbazin medicated	Resistant	20	0	0	0.00	221	99	221	99
Trithiadol medicated	Normal	20	0	0	0.60	217	97	217	97
Trithiadol medicated	Resistant	20	0	0	0.65	198	89	198	89
Zoalene medicated	Normal	19	0	0	0.00	238	107	238	107
Zoalene medicated	Resistant	20	0	0	0.00	244	109	244	109
Nitrofurazone medicated	Normal	20	0	0	0.40	190	85	190	85
Nitrofurazone medicated	Resistant	19	3	16	1.79	126	57	197	88
Arsenosobenzene medicated	Normal	21	0	0	0.00	219	98	219	98
Arsenosobenzene medicated	Resistant	21	0	0	0.00	209	94	209	94
Unistat medicated	Normal	21	0	0	0.00	224	100	224	100
Unistat medicated	Resistant	19	0	0	0.05	219	98	219	98
TRIAL NO. 2									
Uninoculated, unmedicated control	None	20	0	0	0.00	238	100	238	100
Inoculated, unmedicated control	Normal	20	4	20	2.30	80	34	148	62
Inoculated, unmedicated control	Resistant	20	7	35	2.75	52	22	181	76
Nitrofurazone medicated	Normal	20	0	0	0.75	137	57	137	57
Nitrofurazone medicated	Resistant	20	3	15	1.40	87	36	134	56

mately 15 percent mortality in each of the 2 trials in which this drug was used against the drug-fast strain, suggesting that there was a cross-resistance to this drug in the laboratory-induced glycarbylamide-resistant strain of *E. tenella*.

DISCUSSION

Harwood and Stunz (1953) were unable to detect resistance to nitrofurazone in field strains of *E. tenella* and *E. necatrix*. Cuckler and Malanga (1955), however, found resistance to nitrofurazone, nitrophenide, and sulfaquinoxaline, respectively, in certain field strains of these organisms; and, more significantly, they demonstrated cross-resistance to one or more of the drugs. Furthermore, these investigators experimentally induced resistance to sub-optimal dosages of sulfaquinoxaline in one strain of *E. acervulina* and in 2 strains of *E. tenella*. Joyner (1957) reported that a strain of *E. tenella* developed drug resistance after nine passages in chicks fed sub-optimal levels of nitrofurazone. This strain showed no decrease in susceptibility to sulfaquinoxaline or to sulfadimidine. Horton-Smith (1958) described the development of drug-fast strains of *E. tenella* to sulfaquinoxaline and to sub-optimal levels of nitrofurazone, but there was no evidence of cross-resistance in either strain. Horton-Smith also indicated that there was some evidence of a partial and slight resistance to nicarbazin during the sixteenth passage through chickens receiving 0.0125 percent of the drug in the mash.

Although it has been possible to demonstrate drug-fastness in avian coccidia for some time, the physiological and biochemical differences between the resistant and normal strains are not yet known. Limited evidence obtained at this laboratory indicates, however, that the resistant and normal strains are apparently reciprocally immunogenic.

Cross-resistance in field strains of *E. tenella* and *E. acervulina* was demonstrated by Cuckler and Malanga (1955). The cross-resistance to nitrofurazone by the glycarbylamide-fast strain of *E. tenella* herein described, to the best of our

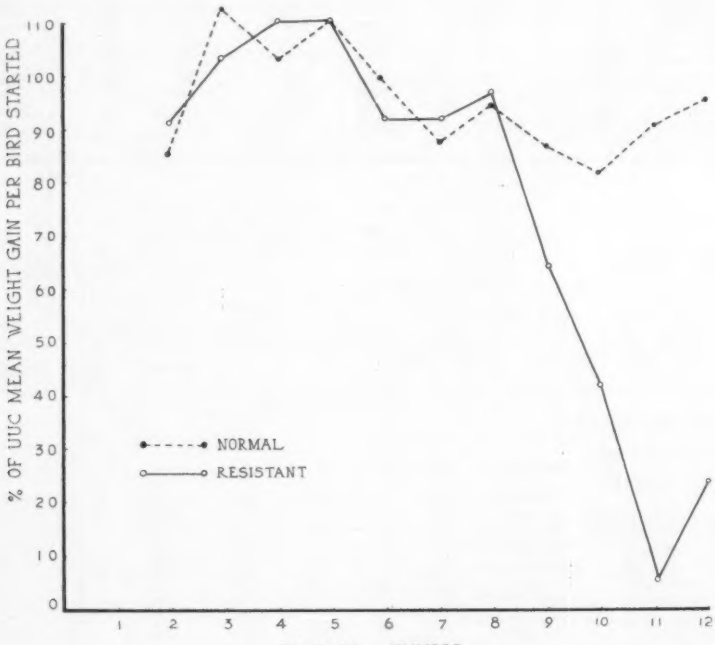

FIGURE 1. Mean weight gain per bird started expressed as percentage of UUC gain of normal- and resistant-line inoculated birds receiving 0.003 percent glycarbylamide.

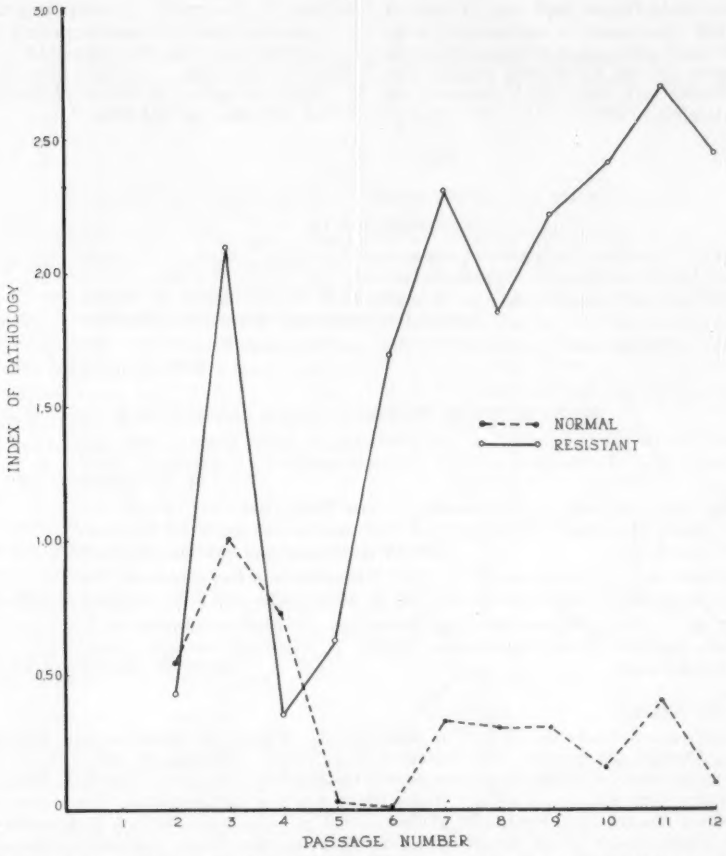

FIGURE 2. A comparison of the index of pathology for normal- and resistant-line inoculated birds medicated with 0.003 percent glycarbylamide.

knowledge, is the first such cross-resistance to be reported for a laboratory-induced drug-fast strain of avian coccidia.

SUMMARY

1. Resistance to glycarbylamide was induced in a laboratory strain of *Eimeria tenella* by repeated exposure to sub-optimal dosages of the drug.

2. Glycarbylamide failed to suppress mortality completely after the ninth passage in chicks given resistant oocysts.

3. The glycarbylamide-fast strain was also resistant to nitrofurazone but not to nicarbazin, Trithiadol, zoalene, arsenosobenzene, or Unistat.

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EFFECT OF SALICYLATES AND RELATED COMPOUNDS ON ABLASTIC RESPONSE IN RATS INFECTED WITH *TRYPANOSOMA LEWISI*. II. DIHYDROXYBENZOIC ACIDS; ANTAGONISMS*

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Tests for possible effects of ortho-, meta-, and para-hydroxybenzoic acids on ablastic response in rats infected with *Trypanosoma lewisi* were previously reported, and ablastic response and antiablastic action defined (Becker, 1961). The study has been extended to include certain dihydroxybenzoic acids, viz, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dihydroxybenzoic acids. The possibility of antagonism between any of these compounds and salicylic acid (ortho-hydroxybenzoic acid), which when administered under an appropriate dosing regimen has an antiablastic effect, was also explored. Meta- and para-hydroxybenzoic acids, isomers of salicylic acid, were also tested for antagonism to salicylic acid. A striking interaction between 2,4-dihydroxybenzoic acid (2,4-DBA) and salicylic acid (SA) was encountered in the tests for antagonisms.

MATERIALS AND METHODS

Parasite, rat host, feeding and handling of the rats, method of drug administration (gavage), and most other procedures were the same as previously (loc. cit.). All the rats were young, weighing from 140 to 280 g when inoculated. Coefficient of variation, the index to intensity of reproduction of the trypanosomes in the previous work, was also employed in some of the present work, but recourse was had in other cases to this less laborious though more subjective method: When the trypanosomes were exceedingly or moderately diverse in size and form and individuals manifestly in a state of division could be located, they rated three asterisks (***); when still somewhat diverse in size and form but reproduction appeared to have ceased, two asterisks (**); when nearly uniform in size and form, and reproduction had obviously ceased, one asterisk (*).

The 2,4-DBA (beta-resorcylic acid) (*see* fig. 1) was from two sources. Park Davis and Company contributed samples of a lot obtained from Distillation Products Industries, Rochester, New York, said to be a highly purified substance suitable for synthetic work. The other supplier was K and K Laboratories, Jamaica, New York, who stated their product had a purity of 95 to 98 percent. The sodium salt of 2,5-dihydroxybenzoic (dihydrate) acid (gentisic acid) (*see* fig. 1) was from K and K Laboratories, who declared the purity to be 98 percent. The 2,6-dihydroxybenzoic (gamma-resorcylic) acid (*see* fig. 1) was also a K and K product whose purity was declared to be 92 to 94 percent. The 3,4-dihydroxybenzoic (protocatechuic) acid (*see* fig. 1) was from California Corporation for Biochemicals Research, Los Angeles. It was "C grade" which, according to the catalog, meant that it was not produced by them, but was the same material offered by other suppliers as "highest purity." The 3,5-dihydroxybenzoic (alpha-resorcylic) acid (*see* fig. 1) was a K and K product whose purity was "95 to 99 percent." The para-hydroxybenzoic acid bore the

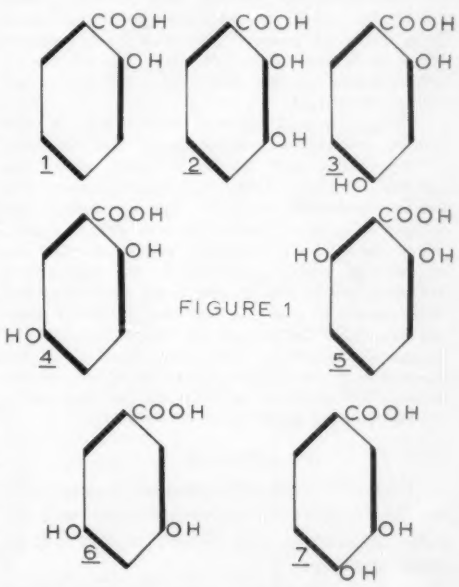

FIGURE 1

Figure 1. Orthohydroxybenzoic and dihydroxybenzoic acids. *1*, ortho-hydroxybenzoic (salicylic; *2*, 2,3-dihydroxybenzoic (not yet tested); *3*, 2,4-dihydroxybenzoic; *4*, 2,5-dihydroxybenzoic; *5*, 2,6-dihydroxybenzoic; *6*, 3,5-dihydroxybenzoic; *7*, 3,4-dihydroxybenzoic acid.

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white label of Eastman Organic Chemicals which meant that it was a chemical of "highest purity" essentially free from isomers, homologs, and impurities. The meta-hydroxybenzoic acid was from K and K Laboratories, who declared its purity to be 95 to 98 percent. The SA (ortho-hydroxybenzoic acid) (*see* fig. 1) bore the white label of Eastman Organic Chemicals, inquiry to whom brought the reply that purity exceeded 98 percent.

As was previously done, sodium bicarbonate in aqueous solution was used to dissolve compounds being tested; e.g., 3.5 g $NaHCO_3$ in 100 ml distilled water dissolved 6 g of SA; 2.8 of it, 6 g of 2,4-DBA. Solutions were made up just before the first administration and stored at 4 C. Crystals separating during refrigeration were redissolved by gentle agitation and heating to 50 C. Solutions slightly opalescent when first made were clarified by filtering through Whatman's No. 1 filter paper. Dosages of drugs are stated in milligrams per 100 g of rat weight.

The infections studied were in rats inoculated with "adult" trypanosomes in diluted blood from untreated passage rats with heavy parasitemias in or beyond the 10th day. The number of trypanosomes injected was roughly 2.0 million per 100 g rat weight.

Wright-stained smears were read in the smooth irridescent zone, between the extremity of the tongue and the thicker part, where the number of erythrocytes was approximately 200 per oil-immersion field. (In case of anemia the number of erythrocytes in this zone was, of course, much less.) The trypanosome count recorded was an average which amounted to the number of trypanosomes in five 200-cell fields. Since the total RBC counts in our rats had been found to average about 8.5 million per cu mm, the number of trypanosomes among 1,000 RBC amounts to the number in about 0.00012 cu mm of blood; or, the number in 1.0 cu mm of blood can be obtained by multiplying the figure recorded by 8,500.

RESULTS

Because the tests for antiablastic action with the five isomers of dihydroxybenzoic acid resulted negatively, only cursory reports will be made of them.

Tests with dihydroxybenzoic Acids for Antiablastic Action

2,4-dihydroxybenzoic acid (2,4-DBA). A student of the author who made thorough tests with this drug for antiablastiic activity found no evidence for it. A few confirmatory data will be presented in the account of tests for antagonism.

2,5-dihydroxybenzoic acid (the sodium salt). Three experiments were conducted involving a total of: 6 infected controls, recipients of distilled water; 6 salicylated controls, recipients of 45 mg of SA daily from the 2d to 7th days, inclusive; and 13 test recipients of 2,5-DBA on the same days in doses ranging from 35 to 60 mg.

The infections in the six normal controls took the usual course with high coefficients of variation for length of trypanosomes on the 3d and 5th days; lower values by the 9th day; and lower still values by the 11th day, when parasitemia persisted. All of the salicylated controls exhibited continued high C.V.s at least 3 days after the 7th day, when the last administration of salicylate took place. The parasitemias in the 13 test rats followed courses very much like those in the normals, with high C.V.s on the 5th and 7th days, lower values on the 9th day, and still lower thereafter so long as the infection persisted. In none of these rats was there the prolongment of the reproductive phase exhibited in the salicylated controls.

2,6-dihydroxybenzoic acid. There were three experiments in each of which there were two normal control rats and one or two salicylated controls dosed as in the previous experiment. The parasitemias in the recipients of 2,6-DBA resembled those in the normal controls rather than those in the salicylated controls.

3,4-dihydroxybenzoic acid. In a single experiment there were two normal controls, one salicylated control, and four test rats administered the test drug at the 45-mg level on the 2d to 7th days. The C.V.s for the test series resembled those of the controls, not those of the salicylate recipient.

3,5-dihydroxybenzoic acid. In a single experiment, there were two normal controls and two salicylated controls. Four rats were dosed with 45 mg of the test drug daily from the 2d to the 7th days, inclusive. The results again resembled those in the previous four experiments.

Tests for Antagonism to Salicylate in Its Antiablastic Role

Antiablastic action is the inhibition of the ablastic response of rats to infection with *T. lewisi;* more specifically, the indefinite prolongment of multiplication of the trypanosome beyond when it would ordinarily have ceased because of the accumulation of (Taliaferro's) antibody ablastin in the blood of the host. Antiablastic action can result from appropriate administration of salicylates (*see* Becker, 1961). In each experiment there were salicylated controls, i.e., rats treated with SA dissolved in solution of $NaHCO_3$ in distilled water, which exhibited the antiablastic response. The rats in

each test series were first administered the same dosage of SA as the controls, and 10 to 15 minutes later the test drug. The drugs tested for antagonism to salicylate were meta- and para-hydroxybenzoic acid and 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dihydroxybenzoic acid. Only one of the isomers and analogs of salicylic acid tested, viz, 2,4-DBA, was found definitely to alter the course of the infection in salicylated rats. Toxic effects were encountered with some of the drug combinations.

Effect of Administration of Both Salicylic Acid (SA) and 24,-Dihydroxybenzoic Acid (2,4-DBA)

The first fifteen experiments bearing on this subject involved a total of 187 rats, and were planned to test effects of 2,4-DBA on the antiablastic action of salicylate. In eight of them were included so-called normal (i.e., infected but not drug-treated) control groups of two to four rats each, and in three there were controls administered 2,4-DBA only. In all of the experiments there were SA recipients and recipients of both drugs.

In every one of the 15 experiments, administration (by gavage) of 2,4-DBA at levels of 35 to 65 mg daily for 6 to 8 days, commencing the day after inoculation, produced a striking effect on the parasitemia in rats dosed beforehand on the same days with 30 to 60 mg of SA. Accounts of three representative experiments follow.

Experiment 1. Ten female rats, from two litters of approximately the same age and

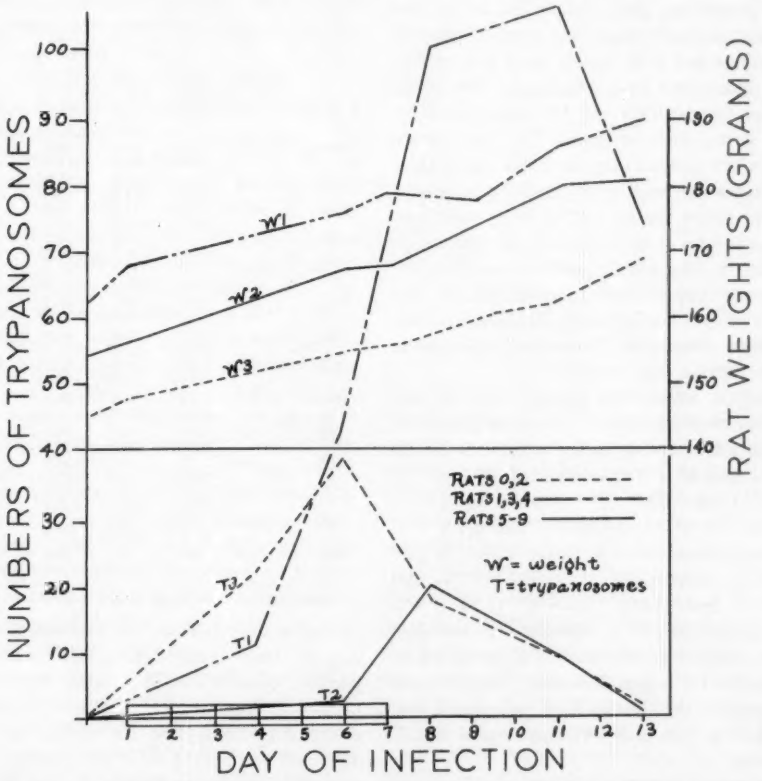

FIGURE 2. Relative numbers of trypanosomes and body weights on various days of *Trypanosoma lewisi* infection for untreated rats (T3, W3), recipients of moderate doses of salicylic acid (T1, W1), and recipients of moderate doses of both 2,4-dihydroxybenzoic acid and salicylic acid (T2, W2) in experiment 1. Drugs administered on boxed-in days (Absolute numbers of trypanosomes per cu mm would be 8,500 times figures indicated in number graphs).

weighing from 147 to 181 g each, were inoculated intraperitoneally at 3:00 P. M. The next morning at 9:00 A. M. two "normal controls" (Nos. 0 and 2) were administered distilled water; three "salicylated controls" (Nos. 1, 3, and 4) received 30 mg of SA; five, the "test series" (Nos. 5, 6, 7, 8, and 9) were dosed in the same manner as the salicylated controls, then 10 minutes later each was given 35 mg of 2,4-DBA. The administrations were repeated daily for six more successive days. Blood smears were made 4, 6, 8, 11, 13, and 18 days after inoculation.

The lower graphs in figure 2 chart relative density of average parasitemias. By 8 days, the parasitemia in the salicylated controls (T1) was near its peak, while in the normal controls (T3) it peaked on the sixth day; the ratio of the two peaks was about 3:1. (The salicylated rats would probably have died by the 10th day if the dosage had been higher, or a day or two later if dosing had been continued.) The striking effect of 2,4-DBA on the salicylated infection is manifest in graph T2, for by the sixth day the average trypanosome population in the test series was approximately 5 percent of that in either the normal or salicylated controls. By 8 days it had risen in the test series and fallen in the normal controls so that the two became approximately equal, but it had mounted in the salicylated controls. Similar results were obtained in three other experiments with the same dosage regimens.

Variation within the groups and asterisk rating of reproduction (in parenthesis) is shown in the following 6-day counts: Normals, 37 (***) and 40 (**); salicylated, 43.5 (***), 55.0 (***), and 35.5 (***); tests, 1.5 (***), 0.5 (***), 2.0 (***), 3.8 (***), and 3.8 (***). The 8-day counts were: Normals, 22.0 (**) and 15 (*); salicylated, 90 (**), 105 (***), and 106 (***); tests, 15 (***), 8 (**), 15 (***), 35 (***), and 26 (***). Intensity of reproduction was rated * in all infections persisting to the 11th day or longer. The differences between the counts for the tests and the salicylated controls on the 6th and 8th days were highly significant.

Experiment 2. The infections in rats receiving both drugs take a markedly different course when the dosages are at considerably higher levels, such as 50 to 65 mg of SA and 60 to 75 mg of 2,4-DBA. The plan and results of this experiment, in which the daily doses of the drug administered 1 to 8 days, inclusive, after inoculation were 50 mg of SA and 60 mg of 2,4-DBA, were representative of six other similar ones conducted within the dosage range stated in the preceding sentence. The parasitemias in the "normal" controls and in the recipients of 60 mg 2,4-DBA ran somewhat parallel courses (*see* table I), as they did in two other experiments. The coefficients of variation in these groups also ran more or less parallel courses for they were high or comparatively high on the 5th and 7th days, and dropped to from 5 to 7.3 percent by the 9th day, and to from 4 to 6 percent by the 11th day. The parasite density in the salicylated controls (Nos. 7 and 8) and coefficients of variation continued high on the 9th day; on the 11th day both animals were found dead. The parasitemias in the test series were much

TABLE I. *Numerical course of the parasitemia and coefficients of variation for lengths of trypanosomes (in parenthesis) in rats of experiment 2 infected with* T. lewisi. *Rats Nos. 1 and 2 "normal controls"; 3, 4, 5, and 6, recipients of heavy doses of 2,4-dihydroxybenzoic acid; 7 and 8, recipients of heavy doses of salicylic acid; 9, 10, 11, and 12, the "test series" receiving both drugs (see text).* (Numbers represent density per .00012 cu mm of blood.)

Rat No. and sex	Days after inoculation 3	5	7	9	11	15
1M	0.1	2.2 (18.1)	20.1 (19.9)	18.0 (5.0)	3 (4)	0.0
2F	0.2	2.9 (17.7)	22.0 (13.3)	19.0 (7.3)	7 (5)	5.0
Mean	**0.2**	**2.6 (12.9)**	**21.0 (16.6)**	**18.5 (6.2)**	**5 (5)**	**2.5**
3M	0.1	1.7 (16.0)	12.6 (19.3)	15.0 (5.8)	6 (5)	0.0
4M	0.0	0.7 (19.5)	11.4 (19.3)	10.7 (5.1)	6 (5)	0.1
5M	0.2	3.1 (15.5)	15.9 (14.2)	16.7 (6.1)	8 (4)	0.0
6M	0.1	2.3 (16.3)	18.9 (18.0)	22.1 (5.5)	11 (6)	4.3
Mean	**0.1**	**2.0 (16.8)**	**14.5 (17.7)**	**16.1 (5.6)**	**8 (5)**	**1.1**
7M	0.3	8.4 (16.6)	39.0 (24.7)	68.0 (19.4)	D	D
8F	0.2	6.3 (19.6)	29.5 (20.9)	93.0 (23.3)	D	D
Mean	**0.3**	**7.4 (18.1)**	**34.3 (22.8)**	**80.0 (21.4)**		
9M	0.0	0.1 (19.6)	2.6 (18.8)	16.6 (23.8)	38 (17)	2.0 (4.5)
10M	0.1	0.8 (13.2)	4.4 (19.8)	37.0 (24.7)	85 (24)	D
11F	0.0	0.2 (12.6)	3.0 (13.4)	38.0 (17.7)	106 (15)	D
12F	0.0	0.4 (19.2)	6.7 (18.0)	54.1 (15.4)	D	D
Mean	**0.0**	**0.4**	**4.2 (17.8)**	**36.4 (20.4)**	**76* (19)**	**2.0* (4.5)**

*, mean of survivors; D, died of the infection.

lower than those in the other three groups on days 3, 5, and 7, but had risen above those in the first two groups by day 9, and continued to increase until the 11th day, when one test host succumbed, and the 15th day, when two more died; one overcame the infection and survived. Coefficients of variation in the test series continued high on the 9th and 11th days.

Low parasite density early in the infection and a tremendous rise after about the 7th day with high coefficients of variation, often culminating in the death of the host, characterized parasitemias in most rats on the higher tolerated dosages of the two drugs.

There were four other experiments in which dosages of intermediate size were employed, viz, 40 mg of SA and 50 mg of 2,4-DBA. The effects of SA and of SA plus 2,4-DBA, as compared with the normal controls, were similar to those obtained with the higher dosages, though not quite so extreme.

Experiment 3. The purpose of this experiment was a direct comparison of a low- with a high-dosage regimen test series. Group 1 consisted of five male rats which received 30 mg of SA and 35 mg of 2,4-DBA the day after inoculation and daily thereafter for 4 days. Group 2, also of five male rats, was on the same regimen except that the dosages of the two drugs were 55 mg and 65 mg, respectively. The rats in both series were able to survive because of the restricted number of drug administrations. The relative mean counts in group 1 on days 3, 5, 8, 11, and 14 were 0, 0.25, 4.2, 1.9, and 0.5, respectively. The same for group 2 were 0.1, 0.26, 37.3, 55.2, and 30.2, respectively. The results confirm the general observation made in the previous 15 experiments that parasitemias in rats on the higher dosage regimen of both drugs regularly mounted dramatically from initial low levels and that only occasionally did they do so when on the lower dosages. In this experiment, however, none of them in rats on the lower dosage levels did so.

Experiment 4. This experiment was undertaken to find out how still smaller dosages of the two drugs than those used previously would affect the parasitemia. There were four "salicylated controls" (2 males and 2 females) administered 20 mg of SA on 7 successive days commencing the day after inoculation. The test group of 10 rats (5 males and 5 females) was on the same regimen except that each rat received in addition to SA 25 mg of 2,4-DBA 15 to 20 minutes after it had been dosed with the 20 mg SA.

The individual and mean counts for the control and treated series are recorded in table II.

TABLE II. *Numerical course of the parasitemia and asterisk rating of intensity of reproduction in rats of experiment 4 infected with* T. lewisi *and administered low dosages of drugs. Rats Nos. 1 to 4, "salicylated controls;" 5 to 14, "test series" (see text).* (Numbers represent density of trypanosomes per .00012 cu mm blood.)

Rat No. and sex	Days after inoculation					
	4	7	9	11	13	23
1M	3.6 ***	46.0 ***	50.0 ***	7.6 ***	5.3 **	0
2F	3.7 ***	53.0 ***	97.0 ***	D	D	D
3F	4.1 ***	48.8 ***	32.0 ***	8.9 ***	11.5 **	0
4M	1.5 ***	18.3 ***	18.8 ***	4.0 **	0.1 *	0
Mean	**3.2**	**41.5**	**49.5**	**6.8**	**5.6**	**0**
5M	0.2 ***	1.9 ***	1.8 *	0.2 *	0	0
6M	1.3 ***	6.8 ***	6.9 **	2.5 *	0	0
7M	0.7 ***	9.8 ***	7.8 **	6.8 *	1.5 *	0
8M	0.3 ***	6.5 ***	4.9 *	3.6 *	0	0
9M	0.4 ***	2.1 ***	3.4 ***	5.1 **	0	0
10F	0.7 ***	9.8 ***	6.6 **	2.1 *	0.3 *	0
11F	1.0 ***	17.5 ***	36.1 ***	65.5 ***	D	D
12F	0.7 ***	9.8 ***	11.9 **	6.8 **	1.5 *	0
13F	0.3 ***	6.5 ***	4.8 **	3.6 *	0	0
14F	0.4 ***	2.1 ***	3.7 ***	5.1	0	0
Mean	**0.6**	**7.3**	**8.8**	**10.1**	**8.5**	**0**

The statistics for the controls on days 11 and 13 are based on three rats because one died on day 10; those for the treated group on day 13 are based on nine, because one died on day 12. At 4, 7, and 9 days the mean counts for the test series were 18.6, 17.5, and 17.7 percent, respectively, of those for the salicylated controls. Fisher's (1930) small-sample test revealed that the differences on each of these days were highly significant. The dosages administered the salicylated controls were in general too small to condition the host for the mounting parasitemia that develop after larger administrations of SA, since three did not develop inordinately high parasitemias and recovered, though one harbored high parasitemia on the 9th day and died on the 10th. One rat (No. 11F) of the test group was unique; it exhibited high parasitemia on the 9th (36.1) and 11th (65.5) days, and was found dead early on the 13th day.

It is to be noted that with three exceptions the intensity of reproduction was rated one asterisk in the test group on day 11.

Another experiment with the same drug regimens employing six normal control rats and six test rats yielded similar results except that no rat developed high parasitemia comparable to that in rat No. 11F, nor were there deaths.

Experiment 5. In this experiment the five salicylated control rats received 10 mg of SA 1 hour after inoculation with trypanosomes and daily thereafter until seven doses in all had been administered. The regimen for the five test rats was the same except that each animal received in addition 12 mg of 2,4-DBA 15 minutes after the administration of the SA. Mean counts with standard deviations for the salicylated controls (upper line) and tests (lower line) 3, 4, 6, and 9 days, respectively, after inoculation were as follows:

7.0 ± 2.5	15.3 ± 16.2	82.4 ± 93.8	68.5 ± 79.6
3.0 ± 2.0	9.0 ± 13.0	44.8 ± 47.9	44.3 ± 59.2

The 17-day counts were fairly even, 28.5 ± 36.7 and 23.2 ± 31.9, respectively. Though the mean counts for the test series were consistently lower, they were not significantly so on any particular day. It appears, however, that the minimal doses of the two drugs required to produce the effect under consideration are close to those employed here.

DISCUSSION

After testing the five dihydroxybenzoic acids, here reported, and para- and meta-hydroxybenzoic acids, previously reported (loc. cit.), salicylic acid and certain of its analogs (*see* Barnes, 1951) remain the only benzoic acid derivatives known to produce the antiblastic effect on parasitemia of *T. lewisi* in rats. The activity of the salicylate molecule appears to depend on the ortho position of the hydroxyl group. Furthermore, introduction of an additional hydroxyl into the molecule at the 4, 5, or 6 position results in loss of the activity. The unlikelihood that 3,4- and 3,5-dihydroxybenzoic acids would posses antiblastic activity was confirmed.

Screening of six benzoic acid analogs for antagonism to salicylic acid in its antiblastic role yielded no positive results. The striking results with 2,4-DBA noted above, however, deserve further consideration.

Is the observed effect a true antagonism? The reproductive phase of the parasitemia is abnormally prolonged and the concentration of trypanosomes in the blood exalted in the properly salicylated host. When, however, the host being salicylated is in turn properly dosed with 2,4-DBA, the increase in the concentration of trypanosomes in the blood proceeds at an abnormally slow rate for a number of days. When relatively heavy doses of the two drugs are employed, a sharp rise in the number curve occurs on the 7th, 9th, or 10th days, but with smaller doses the parasitemia may progress at a low level until its termination. There was a notable individual exception to the latter rule, rat No. 11F of experiment 4 (*see* table II). These low parasitemias consist of morphologically reproductive trypanosomes for about 6 to 8 days; i.e., they exhibit high variability in size and form like those encountered during the corresponding reproductive phase in normal infections or throughout heavily salicylated infections. They assume the adult (i.e., nonvariable and nonreproducing) form, however, before the expiration of the infection when the host survives.

Simple logic would dictate that (1) if antagonism were defined as the partial or total neutralization of the positive action of one factor by a second factor, and (2) if the effect of the second factor were to exceed significantly the point of complete reversal of the first factor, then the observed phenomenon would not qualify as an antagonism. In figure 2, number curve T2 for the test rats may be compared with T3 for the normal (i.e., infected but not drug-treated) rats, and in table I trypanosome counts of test rats 9M to 12F on days 3, 5, and 7 may be compared with those for controls 1M and 2F on the same days. The numbers of rats were small in these series, but in all of eight experiments the means of the counts for normal control rats on days previous to the 9th day markedly exceeded those for rats receiving the double treatment.

It appears that 2,4-DBA of itself has no observable effect on the parasitemia. The phenomenon of the inordinate reduction of the parasitemia by two drugs, one of itself enhancing the trypanosome population by maintaining a continuous high rate of reproduction and the other of itself inactive, therefore, involves something unique. Three speculations regarding the mechanisms responsible follow: (1) The salicylate

renders most of the reproducing trypanosomes susceptible to otherwise non-lethal 2,4-DBA; (2) the two drugs and the rat's body together form a product or products toxic to the trypanosome; (3) since salicylate favors multiplication of trypanosomes, 2,4-DBA has a colchicine-like arresting effect on completion of division of the trypanosomes. There is not much existing evidence for one of these or for any other conceivable hypotheses.

That 2,4-DBA ("BRA") and certain related compounds are capable of activity against lophurae malaria in chickens was reported by Thompson et al (1953), who also found that para-hydroxybenzoic acid ("POB") reverses such activity. No definite indication that either of these two drugs by itself affects *T. lewisi* has been observed in this or previous (loc. cit.) work. If, however, it should subsequensly be shown that SA stimulates the metabolism of that trypanosome, then there would be justifiable grounds for further consideration of the first hypothesis.

The graphs for the mean weights of the three groups of rats appearing in figure 2 are of some interest in that they show that growth may proceed more or less normally under the three different regimens. When, however, as observed in other experiments, considerably higher drug dosages are employed, loss of weight may occur at the time of high parasitemia.

It is premature to speculate whether the observed effect from the reaction in the body of the infected host to the two drugs represents the maximum attainable. Consideration should be given to some of the following with a view to improving the results:

1. Another route of administration of either or both SA and 2,4-DBA, such as the intraperitoneal, intramuscular, subdermal, or intravenous. (It was by gavage in this work.)

2. Reversed order of administering the two drugs. (In these experiments SA was always first.)

3. Other time intervals between the administrations of the two drugs. (In these experiments it was standard procedure to dose with 2,4-DBA 10 to 15 minutes more or less after SA.)

4. Higher and lower ratios of the two drugs in the test animals. (In these experiments approximate salicylate equivalents were employed, according to molecular weights.)

5. Altering frequency of dosings. (In these experiments dosing was diurnal.) Daily administration should be compared with every-other-day and twice-, thrice-, and four-times-daily.

6. Varying the time interval between the time of inoculation and initiation of dosing.

7. Comparison of the behavior of the drugs in *Bartonella*-infected and *Bartonella*-free rats. (Early in the work it had been fairly well established that our infected rats were *Bartonella*-free. Recently *Bartonella* has been seen in heavy infections, though no lice (or fleas) could be found on the rats. When the prolonging effect of pantothenate deficiency on the reproductive phase of *T. lewisi* was first observed, Becker, Manresa and Johnson (1943) were concerned lest the results obtained were to be attributed to resurgent *Bartonella*. Later, however, Becker, Taylor and Fuhrmeister (1947) observed the same prolongment phenomenon in pantothenate-deficient *Bartonella*-free rats. Then Becker and Gallagher (1947) reported prolongment of the reproductive phase and subsequent exaltation of trypanosome density in rats treated with adequate dosings of sodium salicylate; though not so stated, their infected rats were *Bartonella*-free. Recently we have obtained the same effect with salicylate dosing of either *Bartonella*-free or *Bartonella*-infected rats.)

SUMMARY

Certain dihydroxybenzoic acids, viz, the 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-, like para- and meta-hydroxybenzoic acids, but unlike ortho-hydroxybenzoic acid (SA), did not affect the ablastic response in rats infected with *Trypanosoma lewisi*, i.e., the host reaction which inhibits the reproduction of that parasite. In tests for antagonism to SA in its now well-known antiablastic role, only 2,4-dihydroxybenzoic acid was active. Its net effect was the reversal of the activity of SA and, in addition, reduction in the trypanosome population in the blood compared with the "normal," at least for a time. It appears, superficially, that reproduction was not impaired in the reduced population. There was an eventual rise in the parasitemia in rats treated with heavy or moderately heavy doses of SA and 2,4-DBA, but it did not ordinarily occur with comparatively light doses.

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RESEARCH NOTE

COMPETITIONS BETWEEN SUGARS IN THEIR ABSORPTION BY TAPEWORMS.

Recent studies have shown that certain sugars competitively inhibit the absorption of glucose by the vertebrate intestinal mucosa (reviewed by Crane, 1960, Physiol. Rev. **40**: 789–825). In contrast, Phifer (1960, J. Parasit. **46**: 145–153) reported that some of these same sugars have no effect on glucose absorption by the rat tapeworm, *Hymenolepis diminuta*. Since Phifer's experiments on possible competitions in the absorption of sugars by worms were not extensive, this point has been re-examined.

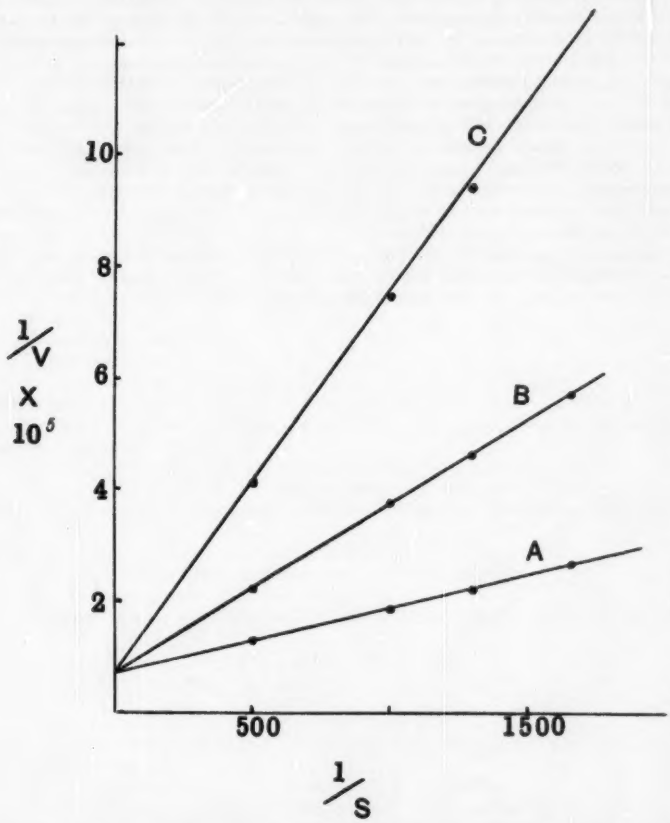

FIGURE 1. Effect of galactose on the uptake of radioglucose by *Hymenolepis diminuta*. Each point is mean of 6 determinations. (V, counts per min. per gram dry weight; S, molar concentration of glucose.) Curve A, glucose only; curve B, glucose plus 0.01 M galactose; curve C, glucose plus 0.02 M galactose.

Hymenolepis diminuta was reared by the usual techniques in male Sprague-Dawley rats, each animal being infected with 30 cysticercoids. Handling of the worms and experimental design were identical with Phifer's (l.c.) in what were termed short interval experiments. Incubations with uniformly labeled glucose-C14 or galactose-1-C14 were of 60-second duration. Extraction, plating, and counting of radioactivity were carried out as described by Read et al (1960, J. Parasit. **46**: 33–41) and Phifer (l.c.). Data were expressed in terms of the alcohol-extracted dry weight after drying the worms to constant weight at 110 C.

Incubation of *H. diminuta* in varying concentrations of radioglucose showed that uptake fitted an adsorption isotherm. The apparent Michaelis constant (Km) was 1.6 mM. Addition of unlabeled galactose inhibited the uptake of radioglucose and analysis of the inhibition showed it to be competitive in nature (fig. 1). Calculation of Km for galactose from its activity as an inhibitor gave values from 5.0 to 5.5 mM. When worms were incubated in radiogalactose at several concentrations, uptake fitted an adsorption isotherm with a Km of 5.1 mM. Uptake of radiogalactose was competitively inhibited by unlabeled glucose.

Experiments of the same design showed that D-allose, alpha-methylglucoside, 6-deoxyglucose, 3-O-methylglucose, and 1-deoxyglucose competitively inhibited the uptake of radioglucose by *H. diminuta*. On the other hand, fructose, L-fucose (6-deoxy-L-galactose), and 1,5-anhydro-D-mannitol had no effect on glucose uptake.

The present observations support the concept that glucose and galactose enter the tissues of *H. diminuta* through a common site. The agreement of the Km values for glucose and galactose uptake as determined by direct observation and by activities as inhibitors indicates that separate special sites for the uptake of glucose and galactose are not present but that these sugars are taken up at the same site. The previous report of Phifer (l.c.) that galactose did not inhibit glucose uptake may be explained by the fact that the differences in glucose and galactose concentrations in Phifer's experiments were not large enough to detect the inhibition. The Km for glucose uptake determined in the present study agrees quite closely with the value reported by Phifer. The sugars taken up through a common site by *Hymenolepis* seem to have in common a structure identical with that of compounds actively absorbed through a common site by vertebrate intestinal mucosa, as described by Crane (l.c.), although the relative affinities of the site for specific sugars are different.

Appreciation is expressed to Dr. Robert Crane for furnishing several of the sugars used. The work was supported by grant E-1384 from the U. S. Public Health Service.—CLARK P. READ, *Department of Biology, The Rice University, Houston, Texas.*

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BOOK REVIEW

Antipin, D. N., Ershov, V. S., Zolotarev, N. A. and Salyaev, V. A. 1960 Parasitology and Parasitic Diseases of Livestock. Moscow English Translation from the Russian by The Israel Program For Scientific Translations for the NSF and USDA, Wash. D. C., 523 pp. Available from the Office of Technical Services, U. S. Dept. of Commerce, Washington 25, D. C., $5.25. Original reference: Ibid. 1956 Parazitologiya i invazionnye bolezni sel'skokhozyaistvennykh zhivotnykh. Moscow, 479 pp.)

This is the second English translation of a Russian book on parasitology produced by the Public Law 480 Program of scientific translations, which is administered by the National Science Foundation (*see* J. Parasit. **47**(2): 196). This textbook on veterinary parasitology was obtained by an American Veterinary Technical Study Group during a visit to Russia in 1958. It is an excellent example of a Russian veterinary textbook, and was of special interest to the Study Group because there was, and still is, a need for an adequate, up-to-date textbook in this field in the United States. This book was translated in Israel by A. Birron, H. G. Hechter and J. I. Lengy.

The technical information in the book is divided into three main sections, i. e., Veterinary Helminthology, Veterinary Arachno-Entomology and Veterinary Protozoology, and is developed in detail mainly on the basis of the diseases produced by various parasites, e. g., fascioliasis, monieziasis, strongylidoses, strongyloidiasis, Mülleriasis, macracanthorhynchiasis, hemosporidioses, etc. A typical breakdown of subheadings under a specific parasitic disease, e.g., dicrocoeliasis, are (a) description of the parasite, (b) development, (c) epizootiology, (d) pathogenicity and pathological-anatomical changes, (e) diagnosis, (f) treatment, and (g) combatting dicrocoeliasis in sheep and cattle. Although the authors obviously have drawn their information for the text from both the world literature and the work of their own veterinary parasitologists, only the names of a few Russian investigators are mentioned in the text. No citations to the literature are given.

The English translation is good, except for an occasional spelling or typographical error, e. g., acanthell(s) for acanthella(s), and all illustrations and tables have been faithfully reproduced from the original, including a number of excellent color plates. No attempt will be made to give a technical evaluation of this textbook, except to say that it appears to give a standard treatment of the biological and clinical aspects of the subject from the Russian point of view and experience; the parasite taxonomy and nomenclature follows precisely the Skrjabin school of thought, and many of the therapeutic and prophylactic treatments recommended appear to be antiquated by our standards.

There is no doubt, however, that teachers and research workers in veterinary parasitology in this country will find this book useful, and will be interested in gaining an insight into the Russian textbook treatment of the subject.—K. C. Kates.

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BOOK REVIEW

Spasskii, A. A. (*Ed.* K. I. Skrjabin) 1961 Anoplocephalate Tapeworms of Domestic and Wild Animals. Essentials of Cestodology, Vol. I. Helminthological Institute, Acad. Sci. USSR, Moscow, 783 pp. (Available from The Office of Technical Services, File No. 61–11490, U. S. Dept. of Commerce, Washington 25, D. C. $8.00. Translation from the Russian). Original reference: Idem 1951. Anoplotsefalyaty-Lentochnye gel'minty domashinikh i dikikh zhivotnykh. Osnovy tsestodologii, Vol. I, Izdatel'stvo Akad. Nauk USSR, Moskva, 735 pp.

This translation of another volume of the K. I. Skrjabin series on Helminthology has been completed under the Public Law 480 Translation Program (*see* J. Parasit. **47**(2): 196, 1961, for brief review of the first volume translated under this program). This book was translated by Dr. A. Birron and Z. S. Cole and was published by the Israel Program for Scientific Translations for the National Science Foundation and the U. S. Department of Agriculture.

This volume was originally recommended for translation by the Beltsville Parasitological Laboratory, ARS, USDA, and the translation manuscript was carefully reviewed by W. W. Becklund of that organization prior to its publication by the Israeli group. All illustrations in the original have been faithfully reproduced in the translation without significant loss of detail. Although a few typographical errors can be found, the translation appears to be excellent.

After a brief preface by K. I. Skrjabin, the book begins with a General Section consisting of a brief Historical Survey and the following chapters pertaining to the suborder Anoplocephalata: (I) Importance of Anoplocephalata in Veterinary and Human Medicine; (II) General Morphological-Anatomical Characteristics; (III) Ontogenetic Stages and Developmental Cycles; (IV) Hosts; (V) Geographical Distribution; (VI) Attempts to Demonstrate Phylogenetic Relationship of Anoplocephalids. Following this is the Systematic Part which covers, species by species, all the cestodes known to the author at the time of its preparation of the superfamily Anoplocephaloidea, with keys to the families, genera and species. The families covered are Anoplocephalidae, Avitellinidae, Linstowiidae and Catenotaeniidae. At the end of the book is a host-parasite list for both adult and larval cestodes of the superfamily, a long list of references (only the Russian are translated), and a good index.

The reviewer believes this monograph is outstanding among the many volumes (about 2 dozen) of the Skrjabin series on helminths, and it is unfortunate that it has been necessary to wait 10 years after original publication for an English translation. It is unlikely, however, that non-Russian specialists on this group will agree with all aspects of Spasskii's systematics, and, undoubtedly, they will point out in future publications areas of disagreement with, and errors in, Spasskii's work. However, I am sure all will agree that this book is an important contribution to helminthology by an outstanding Russian helminthologist.—K. C. KATES.

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